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Editorial policy — The *Journal* publishes original research articles and notes, critical reviews, essays of opinion or hypothesis, letters, and book reviews in the broad field of fisheries management, ocean science, and aquatic environments relevant to Canada. Submissions in English or French are accepted without limitations as to origin, but must not have been published or copyrighted and must not be published elsewhere except in abstract form or by written consent of the Editor. The *Journal* accepts no responsibility for statements made by contributors. Details on submissions are given in guides for authors and typists at the back of this issue. Reprints of these guides are available free from the Office of the Editor.

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Editorial

Over the last few months it has become apparent that the *Journal* is facing a crisis which we can solve only with the understanding and cooperation of our contributors. Rising costs of producing the *Journal*, combined with a rate of submissions that is increasing exponentially, are seriously straining our resources. The recent increase in page charges (from $30 to $40 per page — mandatory for all submissions) covers only a fraction of the higher production costs. A backlog of manuscripts is accumulating but we hope to meet existing commitments through expanded *Journal* issues for November 1976 through March 1977.

Nevertheless, we cannot continue to accommodate the number of manuscripts we receive. Recent federal cutbacks, salary increases, and a high rate of inflation in the printing industry are forcing us to try to limit the quantity and size of manuscripts submitted. We hope to accomplish this by further increasing our standards of acceptance, and by communicating to potential authors through this editorial an elaboration of the more significant aspects of our editorial policy.

Basing the acceptance or rejection of manuscripts on the extent and significance of new knowledge presented has always been our policy; this will continue, but in future must be applied more stringently. A significant proportion of data generated in aquatic science is necessarily purely descriptive. These data are basic to the advancement of science, but cannot be accommodated in primary research journals. The report literature was created specifically for this type of work. The function of the primary journal is to communicate concrete advances in knowledge or theory; widely applicable principles and methodology; and critical, constructive appraisals of broad areas of research. In other words, we deal in synthesis and interpretation rather than in pure facts and description.

Many manuscripts we receive are descriptive, and it would relieve part of our editorial burden if these were submitted to more appropriate journals. We refer particularly to work loosely termed "case history studies." Common topics include patterns of age and growth in specific fish populations; the effects of toxicant A on organism B, or of effluent C on community D; environmental baseline studies; and range extensions that have little zoogeographical significance. We do not denigrate such work, but if no new

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Editorial

Il est devenu évident au cours des quelques derniers mois que le *Journal* est en face d'une crise que seules la compréhension et la coopération de nos collaborateurs nous permettront de résoudre. Des coûts de production à la hausse combinés à un nombre d'articles reçus qui augmente à un rythme exponentiel taxent sévèrement nos ressources. La récente augmentation du taux par page (de $30 à $40 la page—applicable à tous les articles) ne couvre qu'une fraction de l'augmentation des coûts de production. Il s'accumule un arriéré de manuscrits, mais nous espérons pouvoir respecter nos engagements présents en augmentant le volume des numéros du *Journal* de novembre 1976 à mars 1977 inclusivement.

Néanmoins, nous ne pouvons continuer de publier tous les manuscrits que nous recevons. Les récentes coupures budgétaires au fédéral, les augmentations de salaires et le taux élevé d'inflation dans l'imprimerie nous forcent à limiter la quantité et le volume des manuscrits qui nous sont soumis. Nous espérons pouvoir réaliser cet objectif en adoptant des normes d'acceptation encore plus strictes et en exposant aux auteurs éventuels, par le biais du présent éditorial, les aspects les plus importants de notre politique de rédaction.

Notre politique a toujours été d'accepter ou de rejeter un manuscrit selon l'étendue et l'importance des connaissances nouvelles qu'il contient; nous poursuivrons cette politique, mais à l'avenir il nous faudra l'appliquer plus strictement. Une bonne proportion des données qu'engendre la science aquatique sont nécessairement purement descriptives. Ces donnés sont fondamentales à l'avancement de la science, mais ne peuvent trouver place dans des périodiques de recherche de fonds. Des publications ont été créées spécifiquement pour accomoder les rapports sur ce genre de travail. Le rôle d'un périodique de fonds est de communiquer les progrès concrets de la connaissance ou de la théorie; les principes et la méthodologie d'application générale; et les jugements critiques et constructifs dans de vastes segments de la recherche. En d'autres mots, nous nous occupons de synthèse et d'interprétation plutôt que de faits purs et de descriptions.

Un bon nombre des manuscrits que nous recevons sont descriptifs, et si on les soummettait à des périodiques plus appropriés, notre fardeau éditorial serait allégé d'autant. Nous visons particulièrement les travaux vaguement désignés comme «observations de cas particuliers». Sous cette rubrique, on inclut communéinsight or scientific principle is reported the *Journal* will not publish it.

We continue to welcome original, interpretive research articles and notes, reviews, perspectives, and letters to the editor, but we reserve the right to return manuscripts that obviously do not satisfy our requirements without resorting to the normal reviewing process. Also, we may be forced to refer some well-qualified papers to other journals if the submission rate does not drop.

Authors can do much to present their work as concisely as possible. Overly long articles and notes will be edited vigorously in order to maximize the number of items per volume. We will continue to encourage definitive studies by discouraging any apparent duplicate or fragmentary publications. With the continuing cooperation between authors, reviewers, and editors, we hope that the *Journal* will overcome this crisis and maintain its high standards of scientific communication.

ment l'âge et la croissance dans des populations spécifiques de poissons; les effets du toxique A sur l'organisme B, ou de l'effluent C sur la communauté D; des études de référence fondamentale sur l'environnement; et des extensions d'aire qui n'ont pas beaucoup de signification zoogéographique. Nous ne voulons pas minimiser l'importance de tels travaux, mais si un manuscrit ne contient pas de vues ou de principes scientifiques nouveaux, le *Journal* ne le publiera pas.

Les notes et articles scientifiques originaux et interprétatifs, les revues, les essais et les lettres au rédacteur continueront d'être les bienvenus, mais nous nous réservons le droit de retourner les manuscrits qui ne sont pas à la hauteur de nos normes et ne peuvent y satisfaire sans qu'on ait recours au processus normal de révision. En outre, il se peut que nous soyons forcé de transmettre à d'autres périodiques des articles qui se qualifient par ailleurs, si le nombre de manuscrits qui nous sont soumis ne diminue pas.

Les auteurs peuvent nous aider beaucoup en présentant leurs travaux de façon aussi concise que possible. Les notes et articles trop longs seront rigoureusement édités, afin de pouvoir publier un nombre maximum d'item par numéro. Nous continuerons d'encourager la publication d'études définitives en décourageant celle d'études fragmentaires et d'études où il semble y avoir duplication. Que les auteurs, les réviseurs et la rédaction continuent de coopérer, et nous espérons que le *Journal* pourra surmonter cette crise et maintenir sa haute qualité de communication scientifique.

J. C. STEVENSON
Editor and Director of
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l'information scientifique

Announcement

An author–subject index and list of all publications issued during 1976 will be supplied in early 1977, and distributed free to all *Journal* recipients, including subscribers. Additional copies will be available through the Office of the Editor.

Avis

Un index par sujet et auteur et une liste de toutes les publications parues en 1976 paraîtront au début de 1977 et seront distribués gratuitement à tous ceux qui reçoivent le *Journal*, ainsi qu'aux abonnés. On pourra se procurer des exemplaires supplémentaires au Bureau du directeur de la publication.

J. C. STEVENSON
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Physiological Response of Juvenile Coho Salmon (*Oncorhynchus kisutch*) and Rainbow Trout (*Salmo gairdneri*) to Handling and Crowding Stress in Intensive Fish Culture

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WEDEMEYER, G. A. 1976. Physiological response of juvenile coho salmon (*Oncorhynchus kisutch*) and rainbow trout (*Salmo gairdneri*) to handling and crowding stress in intensive fish culture. J. Fish. Res. Board Can. 33: 2699–2702.

Moving 4–5-in. coho salmon (*Oncorhynchus kisutch*) held in soft (20 ppm $CaCO_3$) water from the relatively light loading density of 0.5 lb/ft^3 to 1, 2, or 4 lb/ft^3 (density index, DI = 0.1, 0.2, 0.4, 0.8) caused significant stress as indicated by loss of feeding behavior, but only minimal physiological disturbances, as indicated by lack of hyperglycemia or hypochloremia. However, moving them to 6 or 12 lb/ft^3 (DI = 1.2, 2.4) caused significant physiological stress which required at least a week for recovery. Smolting coho salmon were physiologically stressed by population densities of 1 lb/ft^3 or more and a subclinical corynebacterial kidney infection was activated. Rainbow trout (*Salmo gairdneri*) (4–5 in.) were physiologically stressed when moved and held at 1 lb/ft^3 or more but retained normal feeding behavior. This indicates that handling and crowding stress will be minimized in softwater areas if densities in fish distribution trucks or in ponds or raceways during disease treatments are held to 0.1–0.5 lb/gal.

WEDEMEYER, G. A. 1976. Physiological response of juvenile coho salmon (*Oncorhynchus kisutch*) and rainbow trout (*Salmo gairdneri*) to handling and crowding stress in intensive fish culture. J. Fish. Res. Board Can. 33: 2699–2702.

Le fait de transférer des saumons coho (*Oncorhynchus kisutch*) de 4–5 po. de longueur maintenus dans de l'eau douce (20 ppm de $CaCO_3$) depuis une densité de peuplement relativement faible de 0.5 lb/pi^3 à 1, 2 ou 4 lb/pi^3 (indice de densité, ID = 0.1, 0.2, 0.4, 0.8) produit un stress marqué qui se manifeste par une perte de comportement alimentaire, mais seulement de minimes dérangements physiologiques tels qu'indiqués par un manque d'hyperglycémie ou d'hypochlorémie. Cependant, lorsque transférés à une densité de peuplement de 6 ou 12 lb/pi^3 (ID = 1.2, 2.4), les poissons manifestent un stress physiologique marqué et prennent au moins une semaine à se rétablir. Les saumons coho en train de se transformer en smolts manifestent un stress physiologique à des densités de peuplement de 1 lb/pi^3 ou plus, et le développement d'une infection rénale sublinique. Les truites arc-en-ciel (*Salmo gairdneri*) (4–5 po.) manifestent un

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stress physiologique lorsque maintenues à une densité de 1 lb/pi³ ou plus, mais conservent un comportement d'alimentation normal. Ceci indique que le stress produit par la manipulation et le surpeuplement seront minimisés dans des régions d'eau douce si la densité des poissons dans les camions de distribution ou encore dans les étangs ou dans les passes lors de traitements contre les maladies est maintenue à des densités de 0.1–0.5 lg/gal.

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FISH in intensive culture are continuously stressed by management practices such as handling, crowding, and hauling. All of these, singly or in combination, can impose a considerable load on the somewhat limited homeostatic mechanism of most fishes. Stress requiring a physiological compensation that exceeds the ability of the fish to adjust will be lethal; sublethal stress will compromise ability to tolerate other stressors and will predispose them to infectious or physiological diseases such as myxobacterial gill disease or coagulated yolk (Wedemeyer and Wood 1974).

Crowding stress is particularly important in intensive fish culture and the determination of optimum pond loading densities has received considerable attention in recent years (Piper 1970; Westers 1970; Liao 1971). Most pond loading studies have been directed toward the carrying capacity of the water involved, in terms of dissolved oxygen depletion and ammonia production by the fish. Depending on the water exchange and fish size, population densities (PD) of 5–20 lb fish/ft³ have been reported to be successful for trout (Westers and Leeak 1973). For coho salmon, population densities in the range of 0.5–5 lb/ft³ in standard raceways or deep troughs have been suggested (Burrows and Chenoweth 1955; Wedemeyer and Wood 1974). However, problems still remain, particularly in assessing the amount of stress a particular population density causes and whether or not physiological adaptation to such crowding is possible, and if so, how long it requires.

In addition to rearing fish for extended periods at given pond loadings, fish culturists must sometimes move fish and hold them at increased densities (crowding levels), particularly during such practices as hauling, disease treatments, and pond splitting. Hauling loads are generally 1–2 lb/gal water or about 7–15 lb/ft³ (Smith 1976). The present study was designed to evaluate the severity of the stress caused to rainbow trout (*Salmo gairdneri*) and to coho salmon (*Oncorhynchus kisutch*) by handling followed by crowding, and to determine the length of time these species need for recovery. Blood glucose measurements (hyperglycemia) were used to assess the severity of the physiological stress response (Wedemeyer 1972) and plasma chloride measurements (hypochloremia) to evaluate ionoregulatory dysfunction. Cessation of feeding activity was judged subjectively and used as the behavioral index of the stress response.

Materials and Methods

Groups of 4–5-in. (10–13 cm) rainbow trout (shasta strain) and coho salmon were acclimated to the laboratory water supply (20 ppm total hardness as $CaCO_3$, 10 C) in 4 ft (1.2 m) circular tanks at a density[1] of 0.5 lb fish/ft³, and fed the Oregon moist pellet diet. After 30 days, initial blood samples were taken from 10-fish subgroups by severing the caudal peduncle, and the remaining fish were transferred by dipnet to population densities of 1, 2, 4, 6, or 12 lb/ft³ (DI = 0.1, 0.2, 0.4, 0.8, 1.2, 2.4) at a loading rate of about 1 lb/gpm equivalent to a flow index of 0.2 to insure that the dissolved oxygen remained near 10 ppm. Blood samples were taken from additional 10-fish subgroups at 0 and 4 h after transfer and at 2, 4, 8, 12, and 16 days.

The control fish were not handled or crowded but held at the original acclimation level of 0.5 lb/ft³ and serially sampled as above. Lowering the water level in the circular tank each time a 10-fish subsample was removed maintained the population density at the initial 0.5.

The physiological severity of the resulting crowding stress was estimated by following the occurrence of the hyperglycemia and hypochloremia, using the method of Dubowski (1962) for glucose and standard chloridometer techniques for chloride.

Results and Discussion

When 4–5-in. coho salmon were moved and held at 1, 2, or 4 lb/ft³, they did not suffer any significant blood sugar elevation (Fig. 1), but those held at 6 or 12 lb/ft³ were under a more substantial stress, as indicated by the significant ($P = 0.05$) hyperglycemia which developed within the first 4 h. Partial adaptation to the higher crowding levels occurred within the following 12 days but for fish held at 12 lb/ft³, the blood sugar remained somewhat more elevated

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[1] Loading densities and related data are not given in the metric system because English units continue to predominate overwhelmingly among North American fish culturists. Metric conversion factors are: 1 lb/ft³ = 16 kg/m³; 1 lb/gpm (lb gal^{-1}min^{-1}) = 12 kg·liter^{-1}·min^{-1}.

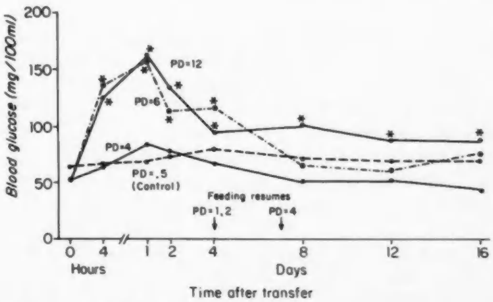

FIG. 1. Sublethal stress response (hyperglycemia) in 4–5-in. coho salmon (*Oncorhynchus kisutch*) transferred from a population density (PD) of 0.5 to 4, 6, or 12 lb/ft^3; fish held at 6 and 12 lb/ft^3 did not resume feeding. * Indicates significant differences ($P = 0.05$). For clarity, PD = 1, 2 are omitted since they were similar to the curve for PD = 4.

than at the lower densities even after 16 days when the experiments were terminated. Together with the hyperglycemia, a significant loss of regulatory precision of blood sugar also occurred at the higher population densities as shown by the marked increase in glucose variability during the first few days (Fig. 1). An increased variance may also imply that some fish regulated poorly while others did not. However, in either case the 6 and 12 lb/ft^3 population densities imposed a significant stress on coho salmon and at least 8 days were required for normalization. The mild hyperglycemia persisting beyond this in the 12 lb/ft^3 group was statistically significant but probably of minor biological significance.

Ionoregulation was apparently not significantly compromised by any crowding level tested as indicated by relatively stable plasma chloride levels. Only a mild hypochloremia developed which was not well correlated with the population density. Similarly, the precision of chloride regulation was not affected as shown by the lack of a significantly increased variability.

Feeding behavior is known to be sensitive to stress, and such proved to be the case in these experiments. The coho stopped feeding immediately upon transfer to all increased population densities and, at the lower crowding levels, did not resume feeding for 4–7 days. At the higher densities of 6 and 12 lb/ft^3, feeding had not actively resumed even after 16 days when the tests were terminated.

Thus, if juvenile coho salmon in soft water must be moved and crowded during fish cultural operations, population densities below 4 lb/ft^3 (DI= 0.8) will reduce stress. For fish distribution trucks, this would be 0.5 lb/gal.

It is sometimes necessary to handle and transfer smolting coho in the course of fish cultural operations. When this was done in these experiments, much more severe blood chemistry disturbances resulted than in the case of parr. As shown in Fig. 2, moving smolting coho (6 in.) from 0.5 to 1, 2, 4, 6, or 12 lb/ft^3 caused significant hyperglycemia in all groups, but it was noticeably more severe in fish held at 2, 4, 6, or 12 lb/ft^3 than in those held at 1 lb/ft^3. Substantial mortalities occurred in the groups crowded at densities of more than 2 lb/ft^3. These proved to be due to activation of an unsuspected latent corynebacterial kidney infection in this lot of experimental fish. There were few mortalities in the fish held at 1 lb/ft^3. Also, there was essentially no resumption of normal feeding over the 16-day holding period at 2 lb/ft^3 or above, but in the 1 lb/ft^3 group, feeding resumed after 10 days. Crowding also caused a general loss of precision in blood sugar regulation in the smolting coho. It was not well correlated with population density but it was generally less severe at 1 lb/ft^3 than at the higher densities (Fig. 2). In contrast to the parr where mild hypochloremia developed, plasma chloride levels in the smolts were not affected by the degree of stress imposed in this study. Ionic regulatory mechanisms might be expected to be more efficient in smolts and this may be correlated with the anadromous life history of this species (Hoar 1976).

For comparative purposes, 4–5-in. shasta strain rainbow trout were subjected to a handling and

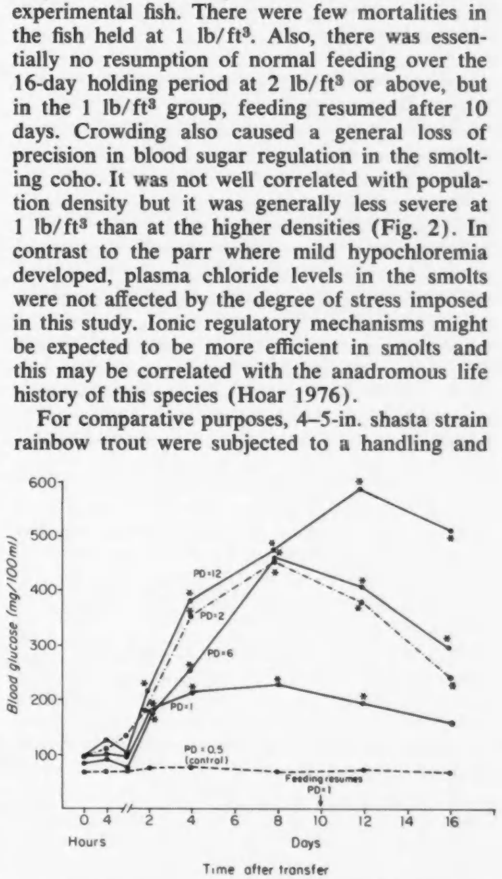

FIG. 2. Stress response (hyperglycemia) in 6-in. smolting coho salmon transferred from a population density (PD) of 0.5 to 2, 6, or 12 lb/ft^3. * Indicates significant difference ($P = 0.05$). Fish held at PD = 2, 6, or 12 lb/ft^3 did not resume feeding. For clarity PD = 4 is omitted since it was similar to PD = 2 and 6.

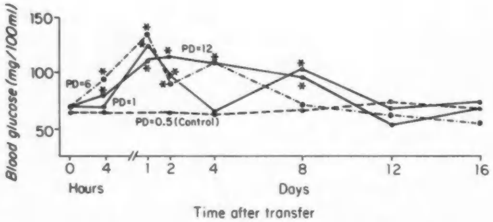

FIG. 3. Stress response (hyperglycemia) in 4–5-in. rainbow trout (*Salmo gairdneri*) transferred from a population density (PD) of 0.5 to 1, 6, or 12 lb/ft^3. For clarity, PD = 2, 4 are omitted since they were similar to the other curves. * Indicates significant difference ($P = 0.05$). Normal feeding resumed in all groups the day after transfer.

crowding regimen similar to that used for the coho parr and smolts. The generalized stress on these fish caused by transferring them from a population density of 0.5 to 1, 2, 4, 6, or 12 lb/ft^3 was indicated by a significant initial hyperglycemia which was about equally severe at all crowding levels (Fig. 3). After the first day the blood sugar levels oscillated somewhat as they gradually returned to their basal values. Loss of regulatory precision of blood sugar followed the same pattern (Fig. 3). Recovery of initial blood sugar levels was essentially complete at all densities within about 12 days. Normal feeding behavior resumed the day after the transfer.

A measurable, but biologically mild hypochloremia also developed which was not statistically significant even at the highest population density of 12 lb/ft^3. However, blood chloride levels did tend to remain depressed longer at this higher loading than at the lower densities. As with the coho salmon, rainbow trout suffered some loss of precision of their chloride regulatory mechanisms but this was also quite mild and not significant statistically.

Previous work has shown that 0.1–0.5% salt additions help alleviate handling and hauling stress but development of additional mitigation procedure was not a part of the present study (Wedemeyer 1972; Wedemeyer and Wood 1974; Hattingh et al. 1975).

In summary, moving juvenile coho salmon, held in soft 10 C water, from the relatively light loading of 0.5 lb/ft^3 (DI = 0.1) to either 6 or 12 lb/ft^3 caused acute sublethal stress. Loadings of up to 4 lb/ft^3 (about 0.5 lb/gal in fish distribution trucks) caused only minimal blood chemistry disturbances. Moving smolting coho to population densities $\gtrsim 1$ lb/ft^3 (0.1 lb/gal in hauling trucks) caused severe stress. Rainbow trout showed significant, but sublethal, stress even at 1 lb/ft^3.

Cessation of feeding behavior by the coho furnished a clinical sign of the underlying physiological stress but rainbow trout resumed feeding almost immediately, showing that observational assessments of fish health can be misleading.

Thus, when crowding of these fishes is necessary, stress must be expected, and a minimum of a week recovery time should be allowed. Though the fish may show normal behavior during this period, additional stress during recovery should be avoided to reduce the load on their homeostatic systems.

Acknowledgments

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BURROWS, R., AND H. CHENOWETH. 1955. Evaluation of two types of rearing pond. U.S. Fish Wildl. Serv. Res. Rep. 39: 29 p.

DUBOWSKI, K. M. 1962. An o-toluidine method for body-fluid glucose determinations. Clin. Chem. 8: 215–235.

HATTINGH, J., F. FOURIE, AND J. VANVUREN. 1975. The transport of freshwater fish. J. Fish Biol. 7: 447–449.

HOAR, W. S. 1976. Smolt transformation: evaluation, behavior, and physiology. J. Fish. Res. Board Can. 33: 1234–1252.

LIAO, P. B. 1971. Water requirements of salmonids. Prog. Fish Cult. 33: 210–215.

PIPER, R. 1970. Know the proper carrying capacity of your farm. Am. Fish. U.S. Trout News 15: 4–7.

SMITH, C. 1976. Fish transportation. *In* J. Bowen [ed.] Manual of fish cultural methods. Part II, section G. U.S. Fish Wildl. Serv. Res. Rep. (In press)

WEDEMEYER, G. 1972. Some physiological consequences of handling stress in the juvenile coho salmon (*Oncorhynchus kisutch*) and rainbow trout (*Salmo gairdneri*). J. Fish. Res. Board Can. 29: 1780–1783.

WEDEMEYER, G., AND J. WOOD. 1974. Stress as a predisposing factor in fish diseases. U.S. Fish Wildl. Fish Dis. Leafl. 38: 8 p.

WESTERS, H. 1970. Carrying capacity of salmonid hatcheries. Prog. Fish Cult. 32: 43–46.

WESTERS, H., AND D. LEEAK. 1973. A comprehensive plan for the Michigan fish hatchery program. Mich. Dep. Nat. Res., Lansing, Mich. 160 p.

Aqueous Leachate from Western Red Cedar: Effects on Some Aquatic Organisms

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PETERS, G. B., H. J. DAWSON, B. F. HRUTFIORD, AND R. R. WHITNEY. 1976. Aqueous leachate from western red cedar: effects on some aquatic organisms. J. Fish. Res. Board Can. 33: 2703–2709.

Water-soluble extractives from western red cedar heartwood, bark, and foliage were investigated for their toxicity to aquatic organisms. The heartwood lignans and bark extractives were found to be moderately toxic, but the foliage terpenes and heartwood tropolones were more toxic, causing 50% mortality to coho salmon (*Oncorhynchus kisutch*) fry at 0.33 and 2.7 mg/liter, respectively. Tropolones were significantly less toxic to invertebrates than to free-swimming stages of the fish tested. Fry were found to be the stage of development of coho salmon (*O. kisutch*) most sensitive to the tropolones, and eyed eggs the least sensitive. Sensitivity of the coho fry to tropolones was moderated by previous sublethal exposure or the presence of a chelatable cation. Results from field studies and a leaching study indicate that directly releasing cedar leachate from landfills or allowing logging debris to enter streams should be avoided.

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Les auteurs ont étudié la toxicité sur des organismes aquatiques d'extraits, solubles dans l'eau, de cœur, d'écorce et de feuillage de cèdre de l'Ouest. Les extraits de lignans de cœur et d'écorce sont modérément toxiques, mais les terpènes du feuillage et les tropolones du cœur sont plus toxiques, causant des mortalités de 50% chez des alevins de saumon coho (*Oncorhynchus kisutch*) à des teneurs de 0.33 et 2.7 mg/litre respectivement. Les tropolones sont significativement moins toxiques pour les invertébrés que pour les stades pélagiques des poissons étudiés. On constate que les alevins sont le stade de développement du saumon coho (*O. kisutch*) le plus sensible aux tropolones, et les œufs embryonnés le stade le moins sensible. La sensibilité des alevins de saumon coho aux tropolones est atténuée par une exposition sublétale antérieure ou par la présence d'un cation chélatable. Les résultats d'études sur le terrain et d'une étude de lixiviation indiquent qu'on devrait éviter le dégagement direct de lessivage de cèdre des remblais ou l'entrée dans les cours d'eau de débris provenant de l'exploitation forestière.

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RECENT restrictions by air quality regulations on the disposal of low quality wood wastes by burning have resulted in the use of increasing amounts of such wood wastes for landfills. Because of the large size and lowland location of many of the landfills, leachates might ultimately enter aquatic environments. Slash remaining after clear-cut logging of western red cedar, *Thuja plicata*, is particularly heavy and may be a potential source of

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leachate. Because little is known about the environmental impact of water-soluble extractives of this species, the purpose of this study was to determine whether these extractives are toxic to aquatic organisms and under what conditions a problem might exist.

Materials and Methods

EXTRACTIVES

Cedar heartwood extract was prepared by extracting 40-mesh wood meal prepared from butt 150- to 400-yr-old heartwood with water at 10 C for 24 h. The solution was filtered and freeze-dried. Tropolones were separated by exhaustive extraction with petroleum ether (20–40 C). The resulting lignans were air-dried, checked for tropolone residue by paper chromatography (Zavarin et al. 1959), and dissolved into a stock solution by warming in distilled water. The tropolones were recovered from the petroleum ether using Kuderna-Danish concentrators and dissolved in 50 C distilled water for use as a stock solution. In addition, β-thujaplicin was obtained commercially from K and K Fine Chemicals, Hollywood, Calif.

The commercial β-thujaplicin, analyzed by paper chromatography, was found to be 95% pure, contaminated by its isomers α- and γ-thujaplicin. Cedar bark extract was prepared from fresh bark by a procedure similar to that used for obtaining the heartwood lignans but omitting the petroleum ether extraction step.

Cedar foliage was steam distilled and the terpenes were recovered by petroleum ether extraction; *l-thujone* and its isomer *d-isothujone* represent 90–95% of the terpene extract (von Rudloff 1962). Stock terpene solutions were made with distilled water.

BIOASSAY TECHNIQUES

The basic bioassay techniques outlined in Doudoroff and Katz (1951) and Sprague (1969) were followed.

Containers — All bioassays were conducted in glass containers held in a water bath in which temperatures were maintained within ±1 C of designated temperature. The tests were static; at least 50% of each test solution was changed every 24 h. The solution volume in the test containers ranged from 1 to 15 liters. Average organism density was about 1 g/liter in most tests. Nonvolatile extractives were tested in open containers and volatile ones in containers covered by sheets of clear 4-mil polyethylene plastic. Dissolved oxygen concentrations were maintained at or near saturation by aeration with compressed air or in covered containers with pure oxygen. Dissolved oxygen, pH, and free CO_2 were monitored regularly throughout the bioassays. Although most bioassays were conducted in barren containers, artificial enclosures and substrates were used in a few selected tests. To facilitate transfer of organisms for water changes, salmon eggs and alevins were held in plastic-screened breeding traps. Small pieces of plastic screen were used as artificial substrates in tests with invertebrates. In the absence of substrates, mayfly nymphs attached themselves to each other, injuring their leg appendages and gill filaments.

Organisms — Most bioassays were conducted with coho salmon, *Oncorhynchus kisutch*. Other organisms used in tests with the tropolone extractives were steelhead trout, *Salmo gairdneri*, prickly sculpins, *Cottus asper*, nymphs of mayflies, *Ephemerella inermis*, and larvae of caddisflies, Limnephilidae. The developmental fish stages included: eggs and sperm during fertilization, eyed eggs, eggs hatching to alevins, alevins, fry (approximately 1 g each), and juveniles (heavier than 3 g). Coho salmon fry or juveniles were also used in bioassays conducted to define the degree of interaction between the toxicity of heartwood tropolone extractives and 1) the pH, temperature, and iron concentration of the dilution water, 2) the genetic stock of the test fish, and 3) the extent to which the fish had had previous laboratory exposure to sublethal levels of those extractives.

Death was the response criterion monitored in all tests. Time of death was defined as follows: fish, cessation of all opercular movements; eyed salmon eggs, acquisition of white coloration; salmon egg fertilization, attainment of development not greater than that attained by unfertilized eggs used as control; and invertebrates, cessation of all undulatory gill filament movements. Ten organisms were used at each concentration of extractive and in the control.

Eggs and alevins were initially measured volumetrically; the exact sample size per concentration (normally near 100) was known only at the completion of each test. Test containers of eggs and alevins were shielded from light by black polyethylene plastic. During all other tests, containers were exposed to cool white fluorescent lighting with a 12-h photoperiod. All tests were conducted for more than 96 h; many were continued in excess of 200 h. The test containers were inspected at 4-h intervals for the test duration. Dead organisms were removed and recorded when first observed.

All invertebrates used in the bioassays were obtained from Woods Creek or Swamp Creek in western Washington. Coho salmon were obtained primarily from either the Quinault National Fish Hatchery, Taholah, Wash., or the University of Washington Hatchery, Seattle. In addition, coho salmon were collected from two streams on the Quinault Indian Reservation, a section of the Olympic Peninsula, Wash., that has recently undergone extensive logging. Because western red cedar is the primary timber species in the watersheds of these two streams, a bioassay was designed to determine whether or not the indigenous salmon had acquired greater tolerance to cedar extractives from this natural exposure. Steelhead trout and prickly sculpins were also obtained from the Quinault Reservation.

Dilution water — All test concentrations were made by mixing the prepared extractive stock solution with an appropriate volume of dilution water.

Tap water, dechlorinated by charcoal filtration, was used as the dilution water in all bioassays and also in all acclimation tanks for organisms. The water was very soft; normal values (milligrams per liter) of total alkalinity, total hardness, and calcium hardness were, respectively: 16, 21, and 17, expressed as $CaCO_3$. The iron concentration ranged from 0.05 to 0.1 mg/liter and the pH from 6.6 to 7.2. Acclimation of test organisms to the water type, temperature, and pH used in subsequent bioassays normally exceeded 10 days.

In the bioassay defining the effects of acidity on the toxicity of heartwood tropolone extractives, the pH of the dilution water was reduced by the addition of hydrochloric acid. A buffer was unnecessary to maintain the pH within the desired range. Ferric chloride ($FeCl_3$) was used as the source of iron for the bioassay designed to define the effect of iron on toxicity.

Data treatment — Cumulative percent mortalities for each concentration were plotted against exposure time on log-prohibit paper by the method described by Bliss (1937). Median survival times (MST) were estimated from the log-prohibit lines and MST vs. concentration curves were plotted on log-log paper for all bioassays in which a series of extractive concentrations were used. The 96-h median lethal concentration (LC50) was determined from each of the plotted MST vs. concentration curves.

Results and Discussion

The water soluble extractives of western red cedar have been extensively studied at the Forest Product Laboratory in Vancouver, B.C. (Barton and MacDonald 1971). The highest concentrations of extractives are found in the heartwood (MacLean and Gardner 1956). Heartwood extractives can be divided into two main groups, lignans and volatiles. The lignans make up 8–18% dry wt of the heartwood and are polyphenolic compounds. Plicatic acid, the major lignan, is a strong organic acid having a pK_a of 3.0. The volatile fraction, 0.5–2% dry wt of the heartwood, consists mainly of tropolones, methyl thujate, thujic acid, and neutrals. The tropolones are known for their fungicidal properties (Erdtman and Gripenberg 1948; Sherrard and Anderson 1933).

LIGNANS

Lignan extractives were only mildly toxic to coho salmon fry and alevins, and even less toxic to mayfly nymphs (Table 1). Because few mortalities occurred during the first 96 h of each test, a determination of the 120-h LC50 was made in lieu of the standard 96-h LC50. No difference was found between the sensitivities of coho salmon fry from the University of Washington Hatchery and the Quinault Hatchery. The LC50 of coho salmon alevins was often significantly greater than those for fry (Table 1). No asymptotic LC50 was indicated for any of the tests with lignans.

TROPOLONE ASSAYS UNDER UNIFORM CONDITIONS

The MST vs. concentration curves obtained from the initial tests of heartwood tropolone extractives with coho salmon fry and mayfly nymphs are presented in Fig. 1. The extractive used was a natural combination of the four major tropolones and thujic acid. The 96-h LC50 was 0.33 and 4.4 mg/liter for coho fry and mayfly nymphs, respectively. The duration of these bioassays was not sufficient to reveal an asymptotic LC50 for the mayfly nymphs. The two organisms may thus have similar toxicity thresholds to tropolones, but the mayflies can definitely tolerate longer exposure before the toxins take effect.

In a bioassay conducted with the single tropolone β-thujaplicin, the 96-h LC50 for coho salmon fry was 0.31 mg/liter (Fig. 2). The MST vs. concentration curve obtained in this bioassay was nearly identical with that in Fig. 1 obtained by testing the natural combination of tropolones and thujic acid with coho salmon fry. Both toxicity curves indicate an asymptotic LC50. In addition, the behavioral responses of the fish were similar in the two tests. Consequently, β-thujaplicin and the composite of the natural tropolones were used interchangeably in subsequent bioassays.

In bioassays with the tropolones (Table 1) an incipient LC50 was observed for all organisms except eyed salmon eggs and the two species of invertebrates. In organisms where an asymptotic LC50 was reached, the MST vs. concentration curves were similar to that obtained for coho salmon fry (Fig. 1). With caddisfly larvae and eyed salmon eggs the MST vs. concentration curves resembled that obtained for the mayfly nymphs (Fig. 1). Sensitivity to the tropolones of early free-swimming stages of the three species of fish tested (Fig. 2) was similar. The highest concentration of tropolone extractive, 2.5 mg/liter, had no significant effect on the survival of eyed salmon eggs over a 240-h exposure. The eyed eggs were clearly less sensitive than the other developmental stages of coho salmon tested. Similar differences have been reported for exposures to zinc (Skidmore 1965), copper (Mount 1968), and malathion (Mount and Stephan 1967). Fertilization of salmon eggs was significantly inhibited when the tropolone concentration in water containing sperm and eggs was 0.7 mg/liter or higher. However, hatching was unaffected

TABLE 1. Results of bioassays using extractives of western red cedar under uniform bioassay conditions.

	96-h LC50 (*mg/liter*)	Organism density (*g/liter*)	Temp. (±1 *C*)
	Heartwood		
Lignans			
Coho fry[a]	64[b]	0.82	10.5
Coho fry[c]	60[b]	0.84	10.5
Coho alevins	33[b]	0.72	10.5
Mayfly nymphs	80[b]	0.05	10.5
Tropolones			
Coho fry[d]	0.33	1.04	10.5
Coho fry[e]	0.31	1.09	10.5
Coho juveniles[e]	0.34	1.79	10.5
Coho eggs/milt[e,f]	0.7[e,g]	420[e,h]	10.0
Coho eyed eggs[e]	>2.5	1.22	10.5
Coho eggs to alevins[e]	1.00	0.84	10.0
Coho alevins	1.05	2.31	10.0
Prickly sculpins[e,h]	0.39	1.81	13.0
Steelhead trout[e]	0.21	0.73	13.0
Mayfly nymphs[g]	4.4	0.10	10.5
Caddisfly larvae[e,h]	7.3	1.70	10.5
	Bark		
Coho fry	28	1.12	10.5
	Foliage		
Coho fry	2.7	1.06	10.5

[a]From Quinault National Fish Hatchery; [b]120-h LC50 in lieu of 96-h LC50; [c]From University of Washington Hatchery; [d]Composite of heartwood tropolone extractives (four primary tropolones and thujic acid) used in this bioassay; [e]β-thujaplicin used in this bioassay; [f]Eggs exposed to milt in a series of extractive concentrations; [g]Concentration of extractive that significantly inhibited fertilization; [h]Egg density during 3-min exposure to milt.

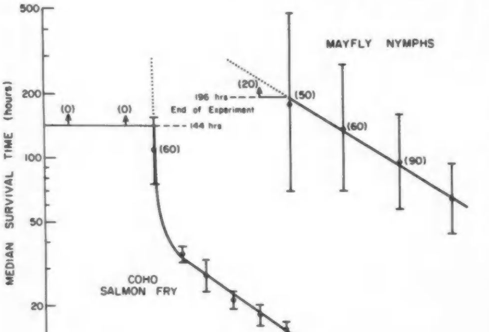

FIG. 1. Toxicity of heartwood tropolone extractives to coho salmon (*Oncorhynchus kisutch*) fry and mayfly nymphs under uniform conditions. The numbers in parentheses are final percent mortalities at the end of the bioassay experiment, if <100.

by concentrations as high as 7.0 mg/liter. Also, the MST vs. concentration curve for the alevins hatched during this bioassay, as subsequently determined, was similar to that obtained previously for alevins.

TROPOLONE ASSAYS UNDER VARIABLE CONDITIONS

Of the various environmental parameters examined, iron concentration in the dilution water had the most significant effect on the toxicity of tropolones to coho salmon. Total iron concentrations of 0.43 mg/liter or higher rendered 1.25 mg/liter solutions of tropolone nontoxic. Tropolones are known to be strong chelators of iron and copper (Barton and MacDonald 1971). Thus, the loss of toxicity is probably the result of a 1:1 chelation between the adjacent hydroxyl–carbonyl of the tropolone ring and iron. Other metal cations such as aluminum and manganese may also chelate with tropolone.

Increased water temperatures shortened the median survival times (MST) of coho salmon exposed to the same concentration of tropolone

TABLE 2. Median survival times of coho salmon (*Oncorhynchus kisutch*) fry from various sources to 1.25 mg/liter β-thujaplicin at 10.5 C.

Source	Organism density (*g/liter*)	Median survival time (*h*)
Quinault National Fish Hatchery	1.17	13.7
University of Washington Hatchery	0.82	14.3
Camp Creek Quinault Reservation	1.21	13.8
Boulder Creek Quinault Reservation	1.18	12.3

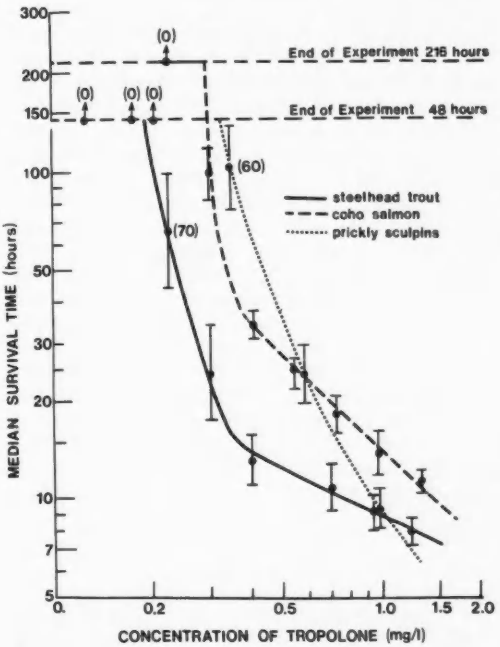

FIG. 2. Toxicity of heartwood tropolone extractives to coho salmon fry weighing about 1 g each (β-thujaplicin was the single tropolone used), steelhead trout (*Salmo gairdneri*) fry (<1 g), and prickly sculpins (*Cottus asper*) (1–3 g). The numbers in parentheses are final percent mortalities at the end of the bioassay experiment, if <100.

extractives. An increase in temperature from 10.5 to 18.5 C lowered the MST from 23.5 to 5.1 h.

There were no significant differences in the sensitivities of four stocks of coho salmon fry to a 1.25 mg/liter concentration of tropolones (Table 2). Thus salmon indigenous to streams within cedar watersheds probably have not acquired adaptive resistance to the tropolone extractive. Coho salmon, held in a 0.23 mg/liter concentration of tropolone extractives (sublethal in all bioassays) for periods in excess of 15 h and subsequently transferred to 1.25 mg/liter of the same extractives, were found to be more resistant to the higher concentration than unacclimated fish. The ultimate resistance obtained by this acclimation procedure was greater than a twofold increase in the LC50. This was achieved by exposures of 60 h or longer to sublethal concentrations. Under these conditions the LC50 was raised from 0.31 to 0.72 mg/liter. Because the bioassay with acclimated salmon was conducted 2.5 C

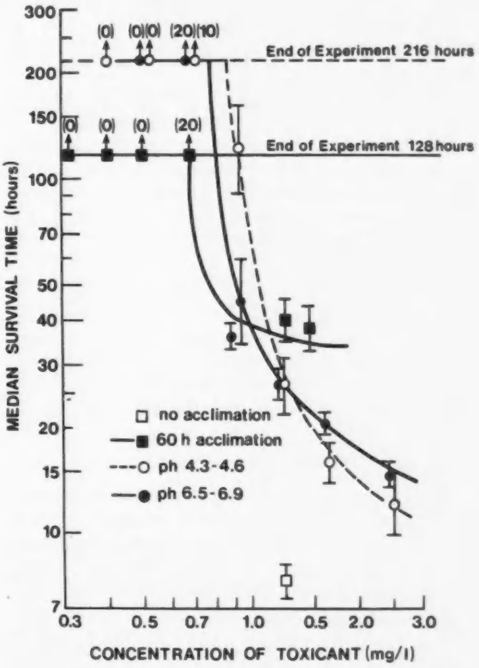

FIG. 3. Effects of pH and acclimation on toxicity of heartwood tropolone extractives to coho salmon fry. The numbers in parentheses are final percent mortalities at the end of the bioassay experiment, if <100.

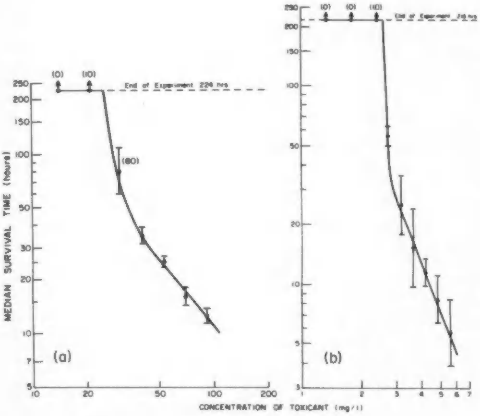

FIG. 4. Toxicity of cedar a) bark extractives and b) foliage terpenes to coho salmon fry. The numbers in parentheses are final percent mortalities at the end of the bioassay experiment, if <100.

higher than with unacclimated fish, the effect of prior exposure may be even greater. This assumption is supported by fivefold difference in the MST of acclimated and unacclimated juvenile coho salmon exposed to a 1.25 mg/liter concentration at the higher temperature (Fig. 3). Because of the short time required for salmon to acquire increased resistance to tropolones during acclimation tests and the uniformity of the reaction times of the four salmon stocks, it is unlikely that the salmon obtained from the two Quinault reservation streams had more than slight natural exposure to tropolones.

Of the environmental parameters investigated, low pH had the least effect on the toxicity of heartwood tropolone extractives to coho salmon. The toxicity curves obtained for the bioassays in acidic (pH 4.3–4.6) and neutral (pH 6.5–6.9) dilution water were nearly identical (Fig. 3).

BARK AND FOLIAGE EXTRACTIVES

Bark extractives were less toxic to coho salmon fry than were the tropolones (Table 1). The MST vs. concentration curve obtained indicates that the asymptotic LC50 may be only slightly less than the 96-h LC50 of 28 mg/liter (Fig. 4a).

Foliage terpene extractives were nearly as toxic as the tropolone extractives of the heartwood to coho salmon fry, having a 96 LC50 of 2.7 mg/liter (Table 1). The MST vs. concentration curve for the terpene was much steeper than that from any other extractive, an asymptotic LC50 being indicated within the first 50 h of the test (Fig. 4b).

Environmental Effects of Cedar Leachate

The toxicity data obtained from the laboratory bioassays indicate that under certain conditions western red cedar leachate could have a significant effect on the aquatic environment. Preliminary information on this effect has been obtained from three cases in which cedar waste leaching was studied (H. J. Dawson unpublished data; Peters 1974).

A laboratory dynamic leaching study was conducted by submerging western red cedar blocks (vol 22.7 liters, surface area 3.2 m^2) in a continuous flow of dechlorinated tap water (14.3 liters/h) (Peters 1974). Bioassays with coho salmon fry were conducted in the effluent water, and several water characteristics were determined, including total organic carbon (TOC), pH, and tropolone concentration. The lignans, which represent the major portion of the TOC, were effectively leached out in the 1st day, and the sharp decrease in pH at the start of this test is at least in part due to the presence of plicatic acid in the leachate (Fig. 5). The concentration of the tropolones remained stable at 0.3–0.4 mg/liter throughout the 85-day test. The leachate water was toxic to the coho salmon fry throughout the test, MST ranging from 18 to 25 h. During the experiment, 2.4 liter of toxic solution/g of cedar heartwood were produced by leaching.

A sample of leachate was obtained from a runoff stream of 2-yr-old cedar waste landfill in the Snohomish River area near Everett, Washing-

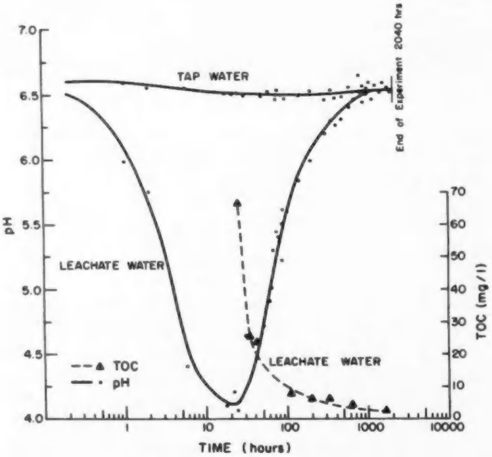

FIG. 5. Values for pH and total organic carbon (TOC) recorded during an 85-day leachate experiment. The pH of tap water before it contacted the cedar block is also indicated.

ton. This sample was intensely colored and had a pH of 4.3 and a BOD_5 (biochemical oxygen demand) of 715 mg/liter. Plicatic acid was determined to be in the 1–2 mg/liter range. Aerated bioassays showed that at a 10:1 dilution the MST for the 10 test fish was 10.4 h; no mortalities were observed at 18:1 and lower dilutions. Tropolone analysis was not performed on the sample.

The effect of logging on forest stream water quality was studied in cedar areas on the Quinault Indian Reservation. Concentrations of tropolones were found in the 0.05 mg/liter in swampy areas and streams in recently logged areas where large quantities of cedar debris were present in the water. Neither tropolones nor lignans were detected in streams that contained older slash or that were adjacent to freshly logged areas where slash was kept out of direct contact with the stream water.

Conclusions

Tropolones were found to be primarily responsible for the toxicity of western red cedar leachate to fish. Leaching of the heartwood with water will result in toxic tropolone concentrations. The toxicity was eliminated by chelation with iron. Leachate toxicity problems could be avoided by the prevention of cedar heartwood and slash contact with streams. This could be accomplished by the use of buffer strips or zones.

Acknowledgments

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BARTON, G. M., AND B. F. MACDONALD. 1971. The chemistry and utilization of western red cedar. Dep. Fish. For., Can. For. Serv., Publ. 1023: 31 p.

BLISS, C. I. 1937. The calculation of the time mortality curve. Ann. Appl. Biol. 24: 815–852.

DOUDOROFF, P., AND M. KATZ. 1951. Critical review of literature on the toxicity of industrial wastes and their components to fish. 1. Alkalies, acids and inorganic gases. Sewage Ind. Wastes 22: 1432–1458.

ERDTMAN, J., AND J. GRIPENBERG. 1948. Antibiotic substances from heartwood of *Thuja plicata* Donn II. Constitution of β-Thujaplicin. Acta Chem. Scand. 2: 625–638.

MACLEAN, H., AND J. A. F. GARDNER. 1956. Distribution of fungicidal extractives (thujaplicin and water soluble phenols) in western red cedar heartwood. For. Prod. J. 6: 510–516.

MOUNT, D. I. 1968. Chronic toxicity of copper to fathead minnows (Pimephales promelas, Rafinesque). Water Res. 2: 215–233.

MOUNT, D. I., AND C. E. STEPHAN. 1967. A method for establishing acceptable toxicant limits for fish-malathion and butoxyethanol ester of 2,4-D. Trans. Am. Fish. Soc. 96: 185–193.

PETERS, G. B. 1974. The effect of leachate from western red cedar, *Thuja plicata* Donn., on aquatic organisms. M.Sc. Thesis. Univ. Washington, Seattle, Wash. 170 p.

RUDLOFF, E. VON. 1962. Gas-liquid chromatography of terpenes VI. The volatile oil of *Thuja plicata* Donn. Phytochemistry 1: 195–202.

SHERRARD, E. C., AND A. B. ANDERSON. 1933. Dehydroperillic acid, an acid from western red cedar. J. Am. Chem. Soc. 55: 3813–3819.

SKIDMORE, J. F. 1965. Resistance to zinc sulfate of the zebrafish (*Brachydanio rerio* Hanilton-Buchanan) at different phases of its life history. Ann. Appl. Biol. 56: 47–53.

SPRAGUE, J. B. 1969. Measurement of pollutant toxicity to fish. I. Bioassay methods for acute toxicity. II. Utilizing and applying bioassay results. Water Res. 3: 793–821.

ZAVARIN, E., R. M. SMITH, AND A. B. ANDERSON. 1959. Paper chromatography of the tropolones of Cupressaceae II. J. Org. Chem. 24: 1318–1321.

Vertical and Horizontal Distributions of Coho Salmon (*Oncorhynchus kisutch*), Yellow Perch (*Perca flavescens*), and Cisco (*Coregonus artedii*) in Pallette Lake, Wisconsin

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ENGEL, S., AND J. J. MAGNUSON. 1976. Vertical and horizontal distributions of coho salmon (*Oncorhynchus kisutch*), yellow perch (*Perca flavescens*), and cisco (*Coregonus artedii*) in Pallette Lake, Wisconsin. J. Fish. Res. Board Can. 33: 2710–2715.

Coho salmon (*Oncorhynchus kisutch*), stocked in Pallette Lake, Wisconsin, remained inshore in April and early May, moved offshore in late May and June, and returned to shore in October. From June to September the vertical distribution of offshore coho salmon overlapped that of yellow perch (*Perca flavescens*) and cisco (*Coregonus artedii*). Yellow perch were gillnetted mainly in epilimnetic water of 16–22 C; coho salmon and cisco were mostly caught in metalimnetic water of 12–17 and 8–12 C, respectively. Vertical separation broke down during other months of the year.

From June to September diel, horizontal movements were found for yellow perch within the epilimnion and for cisco within the metalimnion. Yellow perch moved offshore around sunrise and onshore around sunset; cisco moved toward shore around sunrise and away from shore around sunset. Yellow perch were mainly active during the day and cisco at night. Perch and cisco were, therefore, largely temporally and spatially segregated from each other but not from coho salmon.

ENGEL, S., AND J. J. MAGNUSON. 1976. Vertical and horizontal distributions of coho salmon (*Oncorhynchus kisutch*), yellow perch (*Perca flavescens*), and cisco (*Coregonus artedii*) in Pallette Lake, Wisconsin. J. Fish. Res. Board Can. 33: 2710–2715.

Des saumons coho (*Oncorhynchus kisutch*), ensemencés dans le lac Pallette, Wisconsin, demeurent près du rivage en avril et au début de mai, se déplacent vers le large à la fin de mai et en juin et retournent au rivage en octobre. De juin à septembre, la distribution verticale des saumons coho du large chevauche celle de la perchaude (*Perca flavescens*) et du cisco de lac (*Coregonus artedii*). Les perchaudes sont capturées surtout dans de l'eau épilimnétique de 16–22 C; les saumons coho et les ciscos de lac en grande partie dans des eaux métalimnétiques de 12–17 et de 8–12 respectivement. Cette différence dans la distribution verticale disparaît aux autres mois de l'année.

De juin à septembre, on observe des déplacements horizontaux nychthéméraux de perchaudes dans l'épilimnion et de ciscos dans le métalmnion. La perchaude se dirige vers le large au lever et vers le rivage au coucher du soleil; le cisco émigre vers le rivage au lever et vers le large au coucher du soleil. La perchaude est active surtout le jour, et le cisco la nuit. La perchaude et le cisco sont donc en grande partie séparés, dans le temps et dans l'espace, l'une de l'autre, mais non du saumon coho.

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ANNUAL introductions of coho salmon, *Oncorhynchus kisutch*, into the Great Lakes since 1966 have stimulated interest in stocking them in small, deep lakes for angling. Little detailed information was available on landlocked coho salmon. Foerster and Ricker (1953) found coho salmon in the metalimnion of Cultus Lake, British Columbia. In some midwestern lakes this region is occupied by large yellow perch, *Perca flavescens*, and cisco, *Coregonus artedii* (Hile and Juday 1941; Frey 1955). Concern was, therefore, raised for how coho salmon interact with these native fishes.

The aim of our study was to compare the seasonal depth distributions and diel activity of coho salmon with yellow perch and cisco in Pallette Lake, Wisconsin and to relate these changes to temperature and dissolved oxygen gradients in the lake. The lake was chosen for

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its small size, absence of inlet or outlet streams, and abundance of yellow perch and cisco. Additional studies on food habits, growth, and survival of the fishes have been presented elsewhere (Engel 1972; Engel and Magnuson 1972; Avery 1973).

Materials and Methods

Study Area

Pallette Lake is a seepage lake with a surface area of 69 ha, a maximum depth of 19.3 m, and a mean depth of 9.7 m. It is relatively infertile, with a Secchi disk limit of visibility in summer of 5–6 m and a specific conductance of 24–33 μmho·cm^{-1} at 25 C. Thermal stratification occurs from mid-May to mid-October. Dissolved oxygen reaches 12–13 mg·liter^{-1} in the metalimnion (6–11 m) and 0–2 mg·liter^{-1} in the lower hypolimnion (15–19 m). Among the 17 native fish species in the lake (Engel 1972), yellow perch and cisco are most numerous offshore; yellow perch and common shiners, *Notropis cornutus*, are most abundant inshore.

In October 1968 the Wisconsin Department of Natural Resources stocked the lake with 2500 hatchery-reared coho salmon fingerlings, median total length 117 mm, and in April 1969 with another 2500 fish, median length 137 mm. The groups were hatched in December 1967 and January 1968, respectively.

Inshore Netting and Electrofishing

Three nylon gillnets, 15.2 m wide and 1.8 m deep and with 38-, 51-, and 64-mm stretched mesh, were set parallel to shore in water 2 m deep. Monthly sets were made from August to October 1969 for 4-h periods centered around midday, midnight, sunrise, and sunset.

Fykenetting and electrofishing were done in late April–early May, October and November 1969, and April, May, July, November, and December 1970 (Avery 1973). Fykenets, with a frame 1.2 m on a side and webbing of 9.5-mm stretched mesh, were set at 4–6 inshore locations and lifted after 24 h. Electrofishing was done at night from a boat, using a 230-V, 8.8-A a-c generator. Captured fishes were weighed, measured, and released after sampling.

Offshore Netting

Multifilament nylon gillnets, 5.5 m wide and 18 m deep, were suspended from flotation rollers (Hasler and Tibbles 1970) and lifted from a barge (Engel 1976). Nets were set every 3–4 wk at one offshore station (Station A) from August to November 1968 and at two offshore stations (Stations A and B) from April 1969 to August 1970. In 1968 nets of 38- and 64-mm stretched mesh were used, but in 1969 and 1970 nets of 17-, 38-, and 64-mm mesh were worked at both stations. Nets of 45-, 51-, and 64 mm mesh were added to those at Station B in 1970 to sample coho salmon that had grown larger after stocking. Nets at each station were aligned end to end.

Gillnets were fished for 4–5-h periods centered at midmorning, midafternoon, midnight, sunrise, and sunset. The five periods were randomly distributed over several days with a minimum of 8 h between periods. Catch data from midmorning and midafternoon periods were later combined into a "day" period, because catches were low.

Echo Sounding

Echograms were obtained with two Bendix DR-9 depth recorders having a frequency of 50 kHz and a pulse rate of 178·min^{-1}. Each unit was powered by a 12-V battery having a 205 A·h capacity. Echo soundings were made during each gillnetting period. In 1969 stationary recordings were made from a barge positioned between Stations A and B; in 1970 recordings were made by motoring at about 2 km·h^{-1} across the lake simultaneously in two boats along transects T1 and T2 (Fig. 1). Characteristics of the sound field emitted by the same units were determined by Stone (1961).

Temperature and Dissolved Oxygen Measurements

Water temperature and dissolved oxygen were measured at 1-m depth intervals during the midafternoon period of each monthly gillnetting series. Water temperatures were measured to the nearest 0.1 C using a standardized Whitney electric resistance thermometer. Water samples were collected with a 1.2-liter Kemmerer sampler and analyzed for dissolved oxygen with the sodium azide (Alsterberg) modification of the Winkler method (APHA et al. 1965).

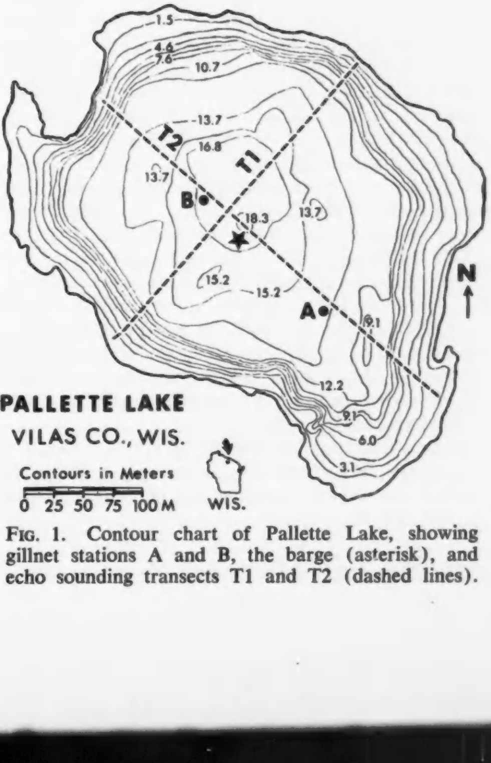

FIG. 1. Contour chart of Pallette Lake, showing gillnet stations A and B, the barge (asterisk), and echo sounding transects T1 and T2 (dashed lines).

Results

Inshore Fish Distribution

Coho salmon were widely dispersed inshore in spring and fall and moved offshore in early summer. Fykenetting and electrofishing inshore captured 468 coho in April and May, 254 from October to December, and 219 in the following April. Electrofishing in July failed to capture any coho and gillnetting inshore from August to October captured only four coho in October. Schools of coho, feeding at or near the surface, were seen during the day from April to June when water temperature ranged from 10 to 18 C, but were not observed from late June to early October when water temperature reached 20–24 C. Offshore gillnetting failed to capture coho in April, November, and December but caught 154 coho from May to October. This suggests a gradual offshore movement of coho in late spring and a return movement to shore during fall overturn.

Yellow perch were abundant inshore throughout the year, schooled during the day, and remained inactive on the bottom at night. Cisco were captured inshore in spring and fall, primarily at night. They spawned in November and December in shallow areas frequented by coho and perch.

Offshore Fish Distribution

Fish distributions (Fig. 2) are based on gillnet catches of 166 coho salmon (165 mm median total length), over 3500 cisco (176 mm), and over 2500 yellow perch (103 mm), as well as more than 525 h of echo sounding. The low numbers of coho captured reflect mainly the low numbers stocked, mortality after stocking, and dispersal into pelagic areas not sampled.

Coho were first gillnetted offshore in May, when they were caught at both stations above 6 m in the epilimnion. Over 90% of gillnetted coho remained in the epilimnion until early July, when they began to move into the metalimnion. By late July and August, with epilimnetic water temperature above 22 C, nearly all coho were found below 6 m in the metalimnion. Coho were not gillnetted in the pelagic region after the start of fall overturn but were captured by fykenetting and electrofishing inshore.

Cisco congregated near the bottom 4 days after ice out in April 1969 (Fig. 2, lower). Echograms revealed a similar concentration of fish targets in 14–16 m water mostly at night. In May cisco were widely dispersed from surface to bottom and echograms revealed fish activity at all depths.

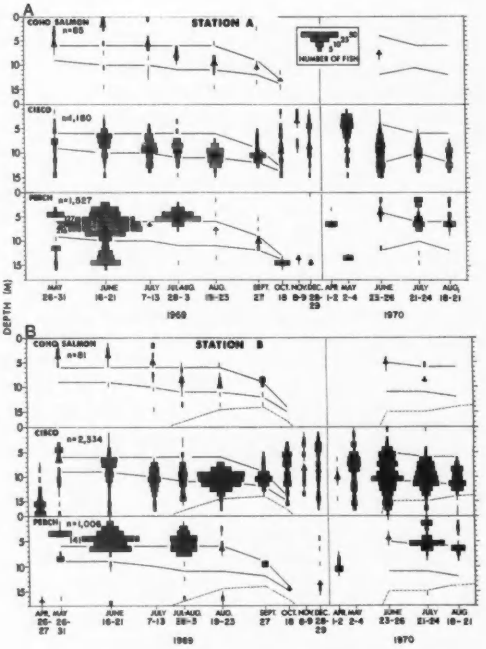

FIG. 2. Depth distributions of fishes gillnetted at Stations A (*upper*) and B (*lower*) in 1969–70. Total number (n) captured is shown for all years. Solid triangles designate median depth of capture; paired solid lines define metalimnion; and broken lines 1 mg $O_2 \cdot liter^{-1}$. Maximum depth at Station A was 15.5 m and at Station B, 18 m.

Beginning in June cisco became progressively concentrated in the lower metalimnion and upper hypolimnion, and only 9% of cisco were gillnetted above 6 m from June to August. Fish traces were particularly concentrated in the metalimnion at night. With onset of fall overturn in October cisco again became widely distributed from surface to bottom.

Yellow perch were not captured in abundance until June and from June to August over 90% of perch were captured at or above 6 m. Fish traces were mostly recorded above 6 m during the day from June to August. At Station A in May (Fig. 2, upper), although many yellow perch were seen near the surface, catch data suggested a broad vertical distribution for perch, mainly because perch near the surface were caught as the net was raised. Few perch were gillnetted after the start of fall overturn, but December sampling suggested a wide vertical distribution. Fish traces under the ice were longer and more widely separated than traces recorded in summer,

Table 1. Diel changes in number of fishes sampled per hour with gillnets at Stations A and B and with echo sounders in 1969–70.[a] Each diel period was 4 h in duration. Total net hours are shown in parentheses and are not multiplied by 10.

	No. sampled	No. · h^{-1} × 10			
		Sunrise	Day	Sunset	Night
1969		(197)	(451)	(242)	(182)
Cisco	2,127	25.0	1.3	33.6	41.8
Yellow Perch	2,139	28.6	25.9	15.2	11.0
Fish traces	4,499	82.9	142.2	140.0	231.0
1970		(161)	(340)	(178)	(192)
Cisco	1,487	25.4	0.7	45.4	56.7
Yellow Perch	431	14.0	4.4	10.8	1.7
Fish traces	4,298	19.4	26.5	23.5	32.6

[a] In 1969 fish traces were recorded from the barge between Stations A and B, and in 1970 along transect T1.

suggesting slower movement and greater fish separation in winter. Hergenrader and Hasler (1968) found similar fish traces in winter from yellow perch in Lake Mendota, Wisconsin.

TEMPERATURE, DISSOLVED OXYGEN, AND FISH DISTRIBUTION

Gillnet catches for midsummer were related to surface water temperatures and low bottom oxygen levels. The median depth of the perch distribution followed the rise in surface temperature during summer to its maximum of 23.5 C in 1969 and 23.7 C in 1970 (Fig. 3). Coho remained in the warmest water until late July, when surface temperature exceeded 18 C, and then moved into cooler metalimnetic water. Cisco were distributed in cooler water and the temperatures at their median depth of capture never exceeded 12 C. From June to September 1969 the temperatures at the median depth of capture were 16–22 C for perch, 12–17 C for coho, and 8–12 C for cisco.

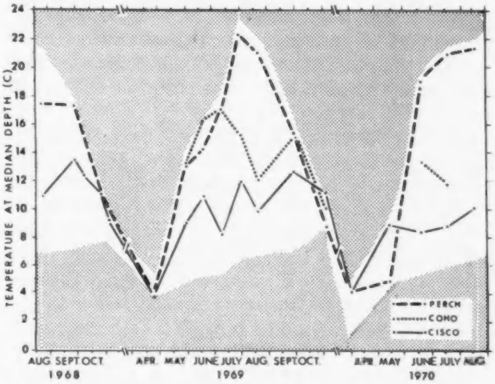

FIG. 3. Temperatures at median depth of fish distributions in gillnets, observed at Stations A and B in 1968–70. Maximum and minimum lake temperatures are bounded by shaded regions.

From July to September the bottom 3–4 m of water contained <2 mg $O_2 \cdot liter^{-1}$. Few fishes were gillnetted or echo sounded in these depths until overturn. Most perch were captured in epilimnetic water containing 7–9 mg $O_2 \cdot liter^{-1}$ and most cisco and coho occurred in metalimnetic water supersaturated with 11–13 mg $O_2 \cdot liter^{-1}$.

DIEL CHANGES IN FISH ABUNDANCE AND ACTIVITY

Catch per unit of effort (CPE) was used as an index to compare fish abundance and activity among diel periods. We defined it as either the number of fish gillnetted per hour of sampling per net, or as the number of fish traces recorded per hour of echo sounding in each 2-m depth interval on the echogram.

For cisco, CPE was 32 times higher at night than during the day in 1969 and 81 times higher in 1970, and was intermediate for sunrise and sunset periods; for perch, in contrast, it was 2–2.5 times higher during the day than at night and was highest at sunrise (Table 1).

Fewer fish traces were recorded during the day than at night from June to September. Large fish traces, representing fish schools, were found in the epilimnion around sunrise and sunset and occasionally during midday. Continuous echo sounding transects revealed an offshore movement of schooling fish around sunrise and an onshore movement around sunset that corresponded in time and depth to perch captured in gillnets.

From June to September a nearly continuous

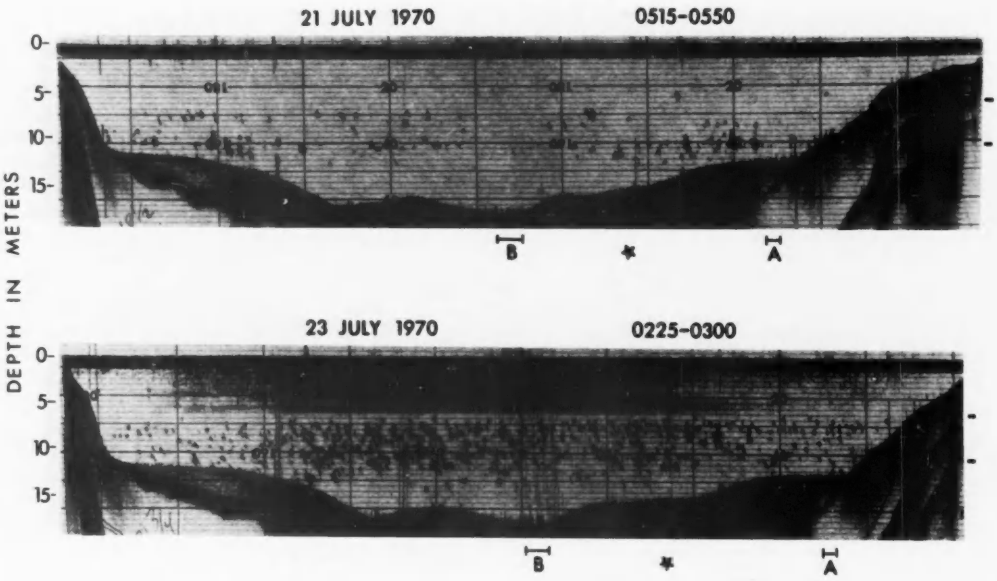

FIG. 4. Day (0515–0550) and night (0225–0300) echograms along transect T2 on July 21 and 23, 1970. *Chaoborus* pupae and larvae produced the dark, shaded band at 0–6 m in the night record. Location of gillnets at Stations A and B and barge (asterisk) are shown under each record. Bars at right delimit metalimnion.

band of fish traces, corresponding in depth and appearance to cisco in gillnets, was recorded throughout the metalimnion at night (e.g. Fig. 4, 0225–0300). Morning and afternoon transects recorded fewer fish traces in the metalimnion and little evidence of fish schooling (Fig. 4, 0515–0550). Continuous transects suggested a progressive disappearance of metalimnetic fish traces from the central pelagic region shoreward, beginning about 30 min before sunrise. Fish traces were more numerous during the day nearshore, where the metalimnion reached the bottom slope at 9–12 m (Fig. 4, 0515–0550). This suggests that cisco congregated near here during the day. A reverse pattern of movement within the metalimnion and toward the central pelagic region was also evident from continuous recordings made around sunset. This offshore movement became evident about 25 min after sunset and resulted in a continuous metalimnetic band of traces within an hour.

Discussion

We conclude that water temperature strongly influenced the seasonal distributions of coho, perch, and cisco and led in part to a partitioning of space in the lake. At no time were the three species isolated from each other. The extent of spatial overlap, however, varied at different times of year. In spring and fall coho coinhabited inshore areas with perch and cisco. In summer coho overlapped first perch in the epilimnion and then cisco in the metalimnion. Overlap between perch and cisco in the pelagic region in summer was particularly restricted, although spatial segregation broke down at other times of year when the lake was homeothermal. In summer segregation of perch and cisco was further reinforced by a diel pattern of activity in which perch used the pelagic region during the day and cisco at night.

Similar thermal distributions have been reported for the three species in other lakes. Introduced coho were found in water of 11–14 C in Columbia River reservoirs (Korn and Smith 1971) and at about 13 C in Parvin Lake, Colorado (Klein and Finnell 1969). Perch were most common above 20 C in several Ontario (Ferguson 1958) and Wisconsin (Hile and Juday 1941) lakes. Cisco were found at 8–10 C in Lake Nipissing, Ontario (Fry 1937) and at 7–10 C in several Wisconsin lakes (Hile and Juday 1941). Our data also agree with those of other studies (Frey 1955; Higgins 1966) in suggesting that cisco avoid bottom water with little or no dissolved oxygen.

Although diel onshore–offshore movements are well known for perch in many lakes (Carlander and Cleary 1949; Hasler and Bardach 1949; Sieh and Parsons 1950; Emery 1973), such movements of nonspawning cisco have never been detailed before. Such movements may help explain low daytime catches of cisco in gillnets in other lakes, which have usually been attributed to gillnet avoidance (Tibbles 1956).

Coho in Pallette Lake ate mainly insects throughout the year and some cisco in late summer (Engel and Magnuson 1972; Avery 1973). Because of the small size of these coho, fish may not have played a larger role in their diet. The restriction of coho and cisco to the metalimnion in late summer, however, suggests that cisco could become vulnerable to predation by larger coho. Since cisco are found in only about 100 lakes in Wisconsin (Frankenberger 1957), salmon stocking of lakes should be done judiciously and in lakes not containing cisco.

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American Public Health Association, American Water Works Association, and Water Pollution Control Federation. 1965. Standard methods for the examination of water and wastewater including bottom sediments and sludges. 12th ed. New York, N.Y. 769 p.

Avery, E. L. 1973. An experimental introduction of coho salmon into a landlocked lake in northern Wisconsin. Wis. Dep. Nat. Resour. Tech. Bull. 69: 8 p.

Carlander, K. D., and R. E. Cleary. 1949. The daily activity patterns of some freshwater fishes. Am. Midl. Nat. 41: 447–452.

Emery, A. R. 1973. Preliminary comparisons of day and night habits of freshwater fish in Ontario lakes. J. Fish. Res. Board Can. 30: 761–774.

Engel, S. 1972. Utilization of food and space by cisco, yellow perch and introduced coho salmon, with notes on other species, in Pallette Lake, Wisconsin. Ph.D. Thesis. Univ. Wisconsin–Madison, Madison, Wis. 259 p.

1976. Movable platform for working rolling gill nets in lakes. Prog. Fish Cult. 38: 23–25.

Engel, S., and J. J. Magnuson. 1972. Ecological interactions between coho salmon and native fishes in a small lake. Proc. Conf. Int. Assoc. Great Lakes Res. 14: 14–20.

Ferguson, R. G. 1958. The preferred temperature of fish and their midsummer distribution in temperate lakes and streams. J. Fish. Res. Board Can. 15: 607–624.

Foerster, R. E., and W. E. Ricker. 1953. The coho salmon of Cultus Lake and Sweltzer Creek. J. Fish. Res. Board Can. 10: 293–319.

Frankenberger, L. 1957. Whitefish and ciscoes — forgotten fish. Wis. Conserv. Bull. 22: 21–23.

Frey, D. G. 1955. Distributional ecology of the cisco (*Coregonus artedii*) in Indiana. Invest. Indiana Lakes Streams 4: 177–228.

Fry, F. E. J. 1937. The summer migration of the cisco, *Leucichthys artedi* (Le Sueur), in Lake Nipissing, Ontario. Univ. Toronto Stud. Biol. Ser. 44, Publ. Ont. Res. Lab. 55: 1–91.

Hasler, A. D., and J. E. Bardach. 1949. Daily migrations of perch in Lake Mendota, Wisconsin. J. Wildl. Manage. 13: 40–51.

Hasler, A. D., and J. J. Tibbles. 1970. A study of depth distribution of perch (*Perca flavescens*) using a rolling gill net. Ber. Dtsch. Wiss. Komm. Meeresforsch. 21: 46–55.

Hergenrader, G. L., and A. D. Hasler. 1968. Influence of changing seasons on schooling behavior of yellow perch. J. Fish. Res. Board Can. 25: 711–716.

Higgins, B. E. 1966. Food selection by the cisco, *Coregonus artedii* (Le Sueur), in Crooked Lake (Noble–Whitley Co.) Indiana. Ph.D. Thesis. Indiana Univ., Bloomington, Indiana. 131 p.

Hile, R., and C. Juday. 1941. Bathymetric distribution of fish in lakes of the northeastern highlands, Wisconsin. Trans. Wis. Acad. Sci. Arts Lett. 33: 147–187.

Klein, W. D., and L. M. Finnell. 1969. Comparative study of coho salmon introductions in Parvin Lake and Granby Reservoir. Prog. Fish Cult. 31: 99–108.

Korn, L., and E. M. Smith. 1971. Rearing juvenile salmon in Columbia River basin storage reservoirs, p. 287–298. *In* G. E. Hall [ed.] Reservoir fisheries and limnology. Am. Fish. Soc. Spec. Publ. 8: 511 p.

Sieh, J. G., and J. Parsons. 1950. Activity patterns of some Clear Lake, Iowa, fishes. Iowa Acad. Sci. 57: 511–518.

Stone, R. C. 1961. Preliminary investigations of winter activity and movements of the yellow perch *Perca flavescens* (Mitchill) in Lake Mendota Wisconsin. M.Sc. Thesis. Univ. Wisconsin, Madison, Wis. 78 p.

Tibbles, J. J. G. 1956. A study of the movements and depth distribution of the pelagic fishes in Lake Mendota. Ph.D. Thesis. Univ. Wisconsin, Madison, Wis. 193 p.

Survival and Propensity for Homing as Affected by Presence or Absence of Locally Adapted Paternal Genes in Two Transplanted Populations of Pink Salmon (*Oncorhynchus gorbuscha*)

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BAMS, R. A. 1976. Survival and propensity for homing as affected by presence or absence of locally adapted paternal genes in two transplanted populations of pink salmon (*Oncorhynchus gorbuscha*). J. Fish. Res. Board Can. 33: 2716–2725.

An experiment was carried out on pink salmon (*Oncorhynchus gorbuscha*) at the Tsolum River, Comox Bay, B.C., comparing an introduced pure donor stock with a hybrid stock created by crossing females from the donor with males of the local residual stock. Progeny of both treatments were raised in gravel incubators and released as unfed fry. Upon return from the ocean, marked fish were recaptured from catch and escapement.

At the fry stage times of emergence differed, but mean fry lengths, weights, and stages of development were the same in both treatments. Survivals from fry to returning (coastal) adult were identical and comparable to that of other years, as was timing of adult migrations.

Large differences occurred in returns to the river. At the hatchery stream the expected relative rate of return of the hybrid was $p = 0.53$, observed $\hat{p} = 0.91$. Returns to the river below the hatchery stream also favored the local genotype, but less strongly so. The results demonstrated that 1) imprinting alone brought back some pure donor stock, 2) addition of the local male genetic complement improved return to the natal river system to a normal level, and 3) the male complement alone was not sufficient to achieve normal accuracy of return to the natal tributary within the system.

"Hybrid" transplants may hold considerable potential for salmon rehabilitation and enhancement, but genetic and managerial implications should be carefully evaluated. Possibilities exist of seriously affecting fitness and abundance of other stocks and ecosystems.

BAMS, R. A. 1976. Survival and propensity for homing as affected by presence or absence of locally adapted paternal genes in two transplanted populations of pink salmon (*Oncorhynchus gorbuscha*). J. Fish. Res. Board Can. 33: 2716–2725.

Nous avons fait une expérience sur le saumon rose (*Oncorhynchus gorbuscha*) dans la rivière Tsolum, baie Comox, C.-B., au cours de laquelle nous avons comparé un stock de donneurs pur avec un stock hybride résultant de croisements de femelles du stock de donneurs avec des mâles du stock résiduaire local. Nous avons élevé la progéniture des deux groupes dans des incubateurs de gravier et l'avons relâchée comme alevins non nourris. A leur retour de l'océan, des poissons marqués ont été recapturés dans les prises et parmi ceux qui s'étaient échappés.

Au stade d'alevin, les temps d'émergement diffèrent, mais les longueurs, les poids et les stades de développement moyens sont identiques dans les deux traitements. La survie de l'alevin à l'adulte retournant à la côte est identique et comparable à celle des autres années, de même que le temps de migration des adultes.

Il y a de grandes différences dans le nombre de poissons retournant à la rivière. Au cours d'eau de la pisciculture, le taux de retours relatif anticipé de l'hybride est de $p = 0.53$, le taux observé $\hat{p} = 0.91$. Les retours à la rivière en aval du cours d'eau de la pisciculture favorisent également le génotype local, mais de façon moins prononcée. Les résultats démontrent que 1) l'impression seule ramène certains individus du stock de donneurs pur, 2) l'addition du complément génétique mâle local a pour effet d'améliorer le retour au réseau fluvial natal à un niveau normal et 3) le complément mâle seul ne suffit pas à réaliser la précision normale du retour au tributaire natal au sein du réseau.

Les peuplements d' « hybrides » peuvent offrir de grandes possibilités de réhabilitation et de rehaussement des stocks de saumons, mais il faudrait évaluer avec soin les implications génétiques et de gestion. Il est possible que la vigueur et l'abondance d'autres stocks et écosystèmes soient sérieusement affectées.

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Pink salmon (*Oncorhynchus gorbuscha*) transplants between river systems have, with few exceptions, been unsuccessful in maintaining themselves following introduction (c.f. Neave 1965). The success of such transplants could, perhaps, be enhanced by incorporation of one set of genes obtained from a locally adapted parent. For example, many transplants have been attempted to strengthen depleted runs. With such residual populations a relatively immense pool of reproductive cells is still available in the males, as compared to those in a similar number of females. What remains of a locally adapted gene pool could therefore be greatly expanded and preserved by utilizing the available males to fertilize large numbers of eggs imported from a donor stock.

Having completed two successful operations at our experimental Headquarters Creek hatchery on the 1968 and 1970 brood-years of the alternate pink salmon cycle (Bams 1972, 1974), we decided to instigate a test in 1971 on the almost depleted local odd-year run and evaluate some of the differences in morphological, physiological, and behavioral characteristics likely to be associated with local genes and bearing, ultimately, on survival. The problem could be approached by simultaneous introductions of a "pure" donor stock and a "hybrid" stock, created by crossing donor females with local (Tsolum) males, in a single dichotomized experiment. Both experimental stocks would be raised in the hatchery, marked, released, and recovered upon return from the ocean. Survivals to the returned adult stage could then be assessed and an attempt made to correlate any difference with effects of local genes.

This paper is concerned primarily with the relative rates of return at the adult stage and several outstanding features observed in near-shore and within-river adult migrations. Additional differences associated with the treatments were evaluated for the following: rates of larval growth and development, time of fry migration, mean lengths, weights and stages of development at emergence, and, at the adult stage, characteristics of migration and morphology. Most of this information is mentioned only briefly, or not at all, in this paper. More extensive treatment and information is available in Bams (1976).

Materials and Methods

Methodology and equipment associated with this experiment were recorded in detail in Bams (1976) and Bams and Crabtree (1976).

The test was carried out at our experimental gravel-incubator hatchery located on Headquarters Creek, a tributary entering the Tsolum River about 24 km (15 miles) upriver from Comox Bay on the east coast of Vancouver Island. One hatchery evaluation test had been completed at this site (Bams 1972) and equipment and techniques were simply extended for this experiment. In the cycle year, 1969, several hundreds of fish spawned in the Tsolum system and only 12 fish entered the hatchery stream.

The donor stream was the Kakweiken River, which drains into the head of Thompson Sound, Knight Inlet, and is 146 km (91 miles) directly northeast from the mouth of the Courtenay River, or at least 209 km (130 statute miles) from headland to headland and by way of Johnstone Strait and Chatham Channel. The Kakweiken recently has had large pink runs which were under investigation by personnel of the Fisheries and Marine Service (Operations), Vancouver, B.C. The Kakweiken pink run peaks somewhat earlier than the Tsolum run, but it overlaps with the latter so that gametes could be collected coincident with the Tsolum fish's reaching maturity. Adult fish were held at both locations until mature. Males were collected by beach seine from the lower Tsolum (133 fish) and from the hatchery weir (an additional 57 fish). Fifty percent of the males used for spawning were stripped twice, on different spawning days, so that a total of only 140 males supplied 211 bags of milt. Gamete collections and transportations were synchronized. Upon arrival at the hatchery, groups of eggs were divided into two nearly equal portions and fertilized simultaneously. Parallel treatments were carried from early incubation in vertical stack incubators, egg picking, and electronic counting through to transfer to 1.2 × 2.4 × 1.2-m (4 × 8 × 4-ft) gravel incubators and completion of larval development. Upon emergence, fry were selectively marked by the removal of the adipose and one or the other ventral fin and released. In addition to the two test groups an additional 120,000 hybrids were raised and released unmarked to provide a "buffer" population to guard against excessive early mortality due to predation.

Differences between treatments were as follows. At the Kakweiken a (late) segment of the run was utilized for the test, whereas the Tsolum males more nearly encompassed the entire run. A proportion of the Tsolum males was used more than once; all Kakweiken males were used only once. The former were also anesthetized prior to spawn-taking during three out of four operations, whereas the latter were not. No known differences in incubator dimensions, materials, gravel composition, and water supply existed between the two treatments, but the brood of each treatment was, of necessity, raised in separate incubators. Fry of the two treatments varied in their mean time of emergence which resulted in varying ratios of numbers marked and released each day. Efficiency of the fry markers, expressed in validly marked fry as a proportion of the total marked, was low initially when fry of only one source were available. It is not likely that any of these differences constituted a serious bias, especially in view of the magnitude of the differences in response as established for the main test results.

On return from the ocean, marked fish were recovered from three general areas: in the commercial

TABLE 1. Means, variances, and rank sums of egg survivals from green to eyed stages of three treatments and four spawning dates. TK = test crosses, KK = test pure donors, TKP = nontest crosses; N = number of pairs, or groups, of eggs as spawned; S = mean survival in %; s^2 = variance.

Spawning day(s)	Source	N	$\bar{S}$	s^2	Rank sums: Samples	Dates	Groups
Sept. 22 (1)	TK	5	71.0	206.1	96.5		
22	KK	5	75.1	737.2	145.5		
22	TKP	6	62.5	837.2	97.0	339.0	
25 (2)	TK	5	86.1	30.8	143.0		
25	KK	5	37.5	791.2	38.0		
25	TKP	6	60.3	1052.6	109.5	290.5	
29 (3)	TK	5	94.2	9.8	235.5		
29	KK	5	91.9	23.8	214.5		
29	TKP	6	91.5	15.9	232.5	687.5	
Oct. 2 (4)	TK	4	92.9	7.7	172.0		647.0
2	KK	4	86.5	196.2	149.5		547.5
2	TKP	6	95.6	6.0	319.5	641.0	758.5
(1)–(4)	TK	19	84.2	402.7			
(1)–(4)	KK	19	72.0	867.2			
(1)–(4)	TKP	24	77.5	687.7			

fishery at interception points along the route of return; at a weir at the entrance to the hatchery (= natal) stream; and from sampling of carcasses following natural spawning in various streams and rivers including the donor stream. The change in ratio of abundance of the two groups from the original ratio in numbers of marked fry as released was analyzed for statistical significance primarily by χ^2-analysis. Parametric testing for significance followed methods given by Sokol and Rohlf (1969); nonparametric methods are by Siegel (1956).

Results

PARENT, EGG AND FRY POPULATIONS

The adult run into the hatchery stream consisted of only 89 males and 42 females and passed its midpoint on September 29 (Fig. 1), which is about 10 days later than the average for the adjacent even-year runs.

Survival of eggs to the eyed stage was extremely variable. High mortality (10% or more) was associated with low rates (0–10%) of visible embryonic development. Survival of unit groups of eggs (trays as spawned) varied from 0.7 to 97.0% and means for treatments by spawning dates from 37.5 to 95.6% (Table 1). These results deviate from our normal experience. Evidently, an uncontrolled factor had prevented fertilization and/or early development in all treatments and during most spawning dates, particularly during the first two (cf. rank sums, Table 1). Further analysis of these data (Bams 1976) demonstrated that the excessive mortality was not associated with the females and we suggested that the "quality" of the milt was responsible.

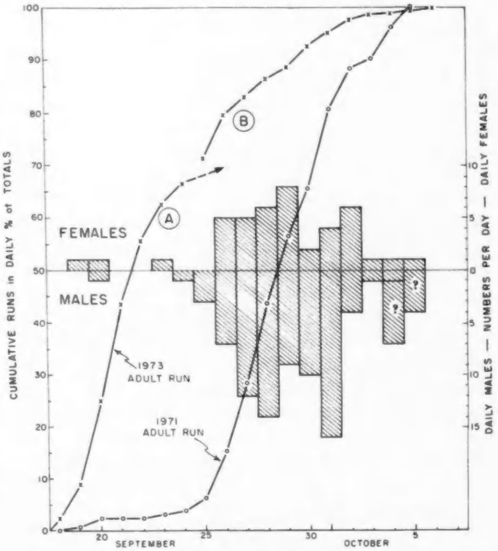

FIG. 1. The 1971 adult pink salmon (*Oncorhynchus gorbuscha*) run into Headquarters Creek in time and by sex. Also given is the 1973 cumulative adult run of unmarked fish only; see text.

Survival in the gravel boxes, from eyed egg to emergent fry, was as follows:

Treatment	Tank	No. of eggs	Fry	S(%)
Test KK	1	121,819	117,220	96.2
Test TK	2	139,682	134,538	96.3
Non-test TK	3	105,657	3,453	3.3
Non-test TK	4	133,928	127,890	95.5

Survival during this period of development was equally high in all three treatments as evidenced by tanks 1, 2, 4. A 2 × 2 χ^2-test on totals of dead and live fry in the two test populations (tanks 1 and 2) shows $\chi^2 = 1.531$, df = 1, $P > .2$. Tank 3 experienced almost total mortality due to a failure of the water supply.

Fry emergence from the boxes started on April 1 for the KK and 3 days later for the TK stock. Rates and changes in rates of emergence were similar in both groups except that the KK group was consistently advanced by about 3 days (Fig. 2). The difference in means (3.0 days) is statistically significant ($P < .001$). The lack of replication leaves a question about homogeneity of experimental environments. Small differences were observed between other tanks which were due to differential mortality prior to planting and to differences in microenvironments available in the different boxes.

Mean lengths, weights, and developmental indices (K_D) of fry migrants were considered to have been the same in both treatments. Marking took place from April 3 to 13 for the KK fry (total valid marks 109,658, mean release date April 5.5) and from April 5 through 13 for the TK fry (total 124,792 and date April 8.2). Calculated mismarks were 4,838 KK and 2,129 TK fry. Additional unmarked TK fry from tanks 2, 4, and 5 brought the total unmarked fry to 144,769 of which 3.3% were of KK origin.

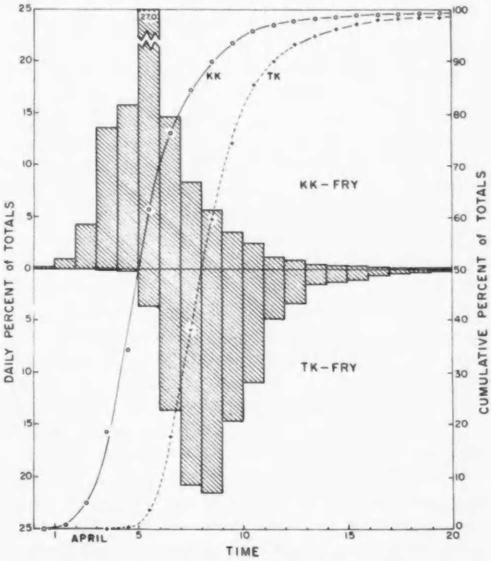

FIG. 2. Daily and cumulative fry runs from the incubator boxes of pink salmon fry of two experimental treatments, KK = pure donor and TK = hybrid stock.

A possible bias was created by differing proportions of mismarks in the two experimental groups. The estimated maximum error in expected relative frequency at return due to this bias was about 0.5%. An error in the final results of similar magnitude was caused by extrapolation of quality-control data which were subject to random variation.

ADULT RETURNS

Recovery of marked fish from the commercial fishery demonstrated equal survival from the fry stage in fish of both treatments. Inspection of the commercial catch began July 6, 1973, in Vancouver and Steveston fish-processing plants. First double marks were found on July 19 and inspections continued through September 8, with the last marked fish recovered on September 5. All recoveries of marked fish and sample sizes are given in Table 2. Initially fish of both treatments were recovered in nearly identical numbers; during the second half of the season the TK fish were consistently somewhat more numerous. However, when cast into a 7 × 2 contingency table, a test for heterogeneity does not yield a significant χ^2-value ($\chi^2 = 2.958$, df = 6, $.9 > P > .75$) and the data are homogenous statistically. Total valid fishery recoveries, 205 KK and 247 TK, were very close to the expected ratio at return (212 and 240), as determined by the ratio at release ($\chi^2 = .435$, $P = .51$). Confidence limits can be estimated for the relative rates of return with a normal approximation to the data (cf. Steel and Torrie 1960). The relative return rate of the TK crosses was .546, the expected rate .531, and their difference .015. Estimated confidence intervals are, at the 95% level .546 ± .046 and at the 99% level ± .060. Clearly, survivals from initiation of migration at the fry stage to interception in the Johnstone Strait area at the returning adult stage were the same in fish from both sources.

Timing was the same for both runs through the fishery. Mean dates of recovery ($\bar{x}$) and other particulars were as follows:

Source	Sex	$\bar{x}$	SD	N
TK	♂	Aug. 8.1	11.4	107
TK	♀	Aug. 11.8	9.4	138
TK	Σ	Aug. 10.2	10.4	247
KK	♂	Aug. 7.2	11.9	91
KK	♀	Aug. 10.8	8.5	113
KK	Σ	Aug. 9.1	10.3	205

There is a suggestion that the KK fish were 1 day ahead of those of the other treatment, which would be consistent with a similar, but

TABLE 2. Recoveries of validly marked adult pink salmon (*Oncorhynchus gorbuscha*) from fish canneries and rivers during 1973.

		Source		
Date, to	Location	KK	TK	Sample size
July 22	canneries	21	22	26,000
29	canneries	14	13	54,000
Aug. 5	canneries	36	36	77,000
12	canneries	56	75	95,000
19	canneries	39	46	40,000
26	canneries	32	41	74,000
Sept. 2	canneries	5	14	156,000
9	canneries	2	0	111,000
Totals	canneries	205	247	633,000
1973	HQ-weir	10	104	426
1973	Tsolum R.	35	124	1,601
1973	Morrison Cr.	0	8	290
1973	Kakweiken R.	0	0	1,100
Totals	Rivers	45	236	3,417
Totals	canneries and rivers	250	483	636,400

TABLE 3. Fence entry records for marked KK, marked TK, and unmarked (NM) adult pink salmon.

				Quartile			
Source	N^a	Mean entry date[b]	SD	1	2	3	D[c]
KK ♂	5	20.0	(1.2)	19.1	19.8	20.4	(0.3)
TK ♂	55	21.0	2.2	19.0	20.0	21.7	1.1
NM ♂	159	23.3	3.7	20.2	21.3	25.4	2.0
KK ♀	5	20.2	(0.4)	19.3	19.6	19.9	(0.6)
TK ♀	49	20.4	1.5	18.9	19.7	20.6	0.8
NM ♀	153	23.6	4.2	19.7	21.7	25.7	1.9
Σ KK	10	20.1	0.9	18.3	18.7	20.2	1.4
Σ TK	104	20.8	1.9	19.0	19.8	20.9	0.9
Σ NM	312	23.4	3.9	20.0	21.5	25.4	1.9

[a] Number of fish in category.

[b] All dates are in September.

[c] Difference between mean and median, apparent discrepancies are due to rounding in earlier columns.

larger, difference in timing observed at the fry stage. However, the difference is statistically not significant ($P > .05$). The cumulative timing data clearly show that the great similarity between the two runs and the consistent but slight advance of one over the other (Fig. 3). Males passed, as is usual (Bams 1972, 1974), significantly earlier through the fishery than the females; in the TK fish the difference between mean dates was 3.7 days, $t = 2.783$, df $= 243$, $P < .01$ and in KK fish, 3.6 days, $t = 2.326$, df $= 202$, $P \approx .01$.

Adult fish congregated in the Courtenay River at the confluence of Puntledge and Tsolum rivers in late August. The first fish arrived at the weir in the hatchery stream on September 18, which was 7–8 days later than has been observed on three successive cycles (1968–72) of the even-year run and 1 day earlier than the 1971 parent run. Various aspects of the adult run are recorded in Table 3.

At the weir the ratio of TK to KK returns deviated greatly from the expected, about 10:1 as opposed to 1:1, or, more precisely, to 10.40 from 1.14. Expressed as a binomial probability, $\hat{p}$, we obtained, for KK, 0.088 instead of 0.468. This difference is highly significant, $\chi^2 = 39.27$, df $= 1$, $P < .001$. Approximate confidence limits

TABLE 4. Proportions of fish of different origins in samples of spawners from different locations along the Tsolum River.

Location[a]	N[b]	KK	%[c]	TK	%[c]	$\hat{p}_{KK}$[d]
HQ	426	10	2.3	104	24.4	.088
A	479	13	2.7	70	14.6	.157
B	400	10	2.5	35	8.8	.222
C	463	6	1.3	17	3.7	.261
D	295	6	2.0	2	0.7	.750

[a] HQ = hatchery weir; A to D = areas of Tsolum successively farther downstream.
[b] N = number of fish in total sample.
[c] % = proportion of fish in preceding column in total sample.
[d] p = binomial probability of KK fish among (KK + TK) fish.

of $\hat{p} = 0.088$ at $P = .975$ are 0.048 to 0.145 (Steel and Torrie 1960, table A.14 B).

Differences in distribution along the entire river system between fish of the two treatments were evident. Firstly, a proportion of fish of both treatments "strayed" in the sense that they did not come upriver far enough to enter the stream of birth. The Tsolum River below the hatchery stream yielded 35 KK and 124 TK marks (Table 2), for a frequency $\hat{p} = 0.220$ for KK fish instead of the expected 0.468. Confidence limits of this probability at $P = .975$ are .157 to .295. Secondly, for areas above and below the weir the rates of return differed significantly. This is supported by a 2 × 2 χ^2-analysis: $\chi^2 = 7.52$, df = 1, $P < .01$. Thirdly, within the main river a gradual shift in relative abundance could be demonstrated. By dividing the main Tsolum below the hatchery stream into sections yielding approximately equal numbers of recoveries, five geographic divisions are obtained in the returns descending from the hatchery stream to the downstream end of the Tsolum, labeled HQ to D on Table 4. A pronounced change in relative abundance of the KK fish ($\hat{p}_{KK}$), is evident as a perfect correlation with a low probability of chance occurrence by Kendall's rank-correlation test, $\tau = 1.0$, $P < .01$. Relative abundance, as shown by percent occurrence in each sample (Table 4), demonstrates that the progression is caused by decreasing abundance of TK fish while KK fish remained constant over all sections. Evidently TK fish showed a strong preference for the natal stream within the Tsolum system whereas the KK fish did not.

Additional surveys were made in Morrison Creek, a tributary of the Puntledge River, where an unexpected 8 TK but no KK marks were found among 290 carcasses; in Oyster River with no marks in about 100 fish; and in Kakweiken River, the donor stream, where no marks were found among about 1,100 spawned-out fish. The latter sample is small in relation to the size of the Kakweiken run, estimated at 300,000 fish (Department of the Environment, Fisheries and Marine Service, Vancouver, F381 file reports for 1973) but it was collected when most marks could perhaps be expected, based on time and location of the parent fish taken 2 yr previously.

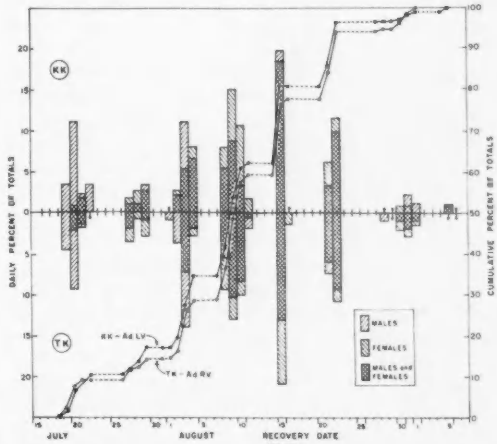

FIG. 3. Daily and cumulative recoveries of marked adults of two treatments, TK and KK, from the commercial fishery. The symbol ⚲ signifies zero recoveries on days that fish were available; the symbol | indicates that no fish were available for sampling.

Passage of unmarked fish through the hatchery weir (Table 3 and Fig. 4) differed markedly from that of the known TK fish. Mean dates differed by 2.6 days, $t = 9.0$, $P < .001$, and variances of the means also differed significantly $F = 4.33$, df (311, 103), $P < .001$. There is a suggestion that we are dealing with two populations here. The cumulative curve of Fig. 4 shows an early part (A), comprising about 70% of the run, which may consist primarily of unmarked TK fish produced by the hatchery, and a later part (B), the remainder of the run, consisting of

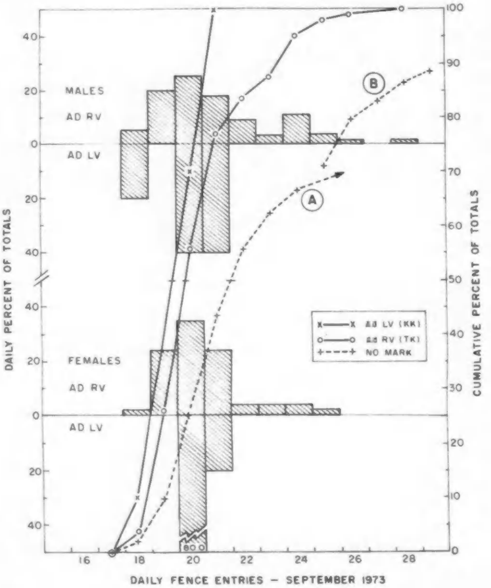

FIG. 4. Daily and cumulative runs of marked adults of two treatments, TK and KK, through the hatchery weir. The cumulative run of unmarked adults is also given; see text.

TT fish which were produced naturally below the hatchery weir and ascended through the weir upon return. The timing of these surmised subruns coincides with the known TK and the parent TT runs (Fig. 1 and 4). This material and the observations on adult lengths, weights, fecundity, and related statistics are treated more extensively in Bams (1976).

Survival rates from fry to adult are known only for marked fish that returned to the hatchery stream; all others can only be estimated. Estimates or rates of survival and marking mortality are of interest in comparison with similar estimates on other marked and unmarked populations (e.g. Parker et al. 1963; Parker 1964; Bams 1972, 1974). The following assumptions are made. Based primarily on experience gained over several years of recovery programs (Bams 1972, 1974) seemingly reasonable estimates of mark-recovery efficiency are .5 for the fishery and .6 for the Tsolum River. The number of unmarked fish that strayed into the hatchery stream is set equal to the number that remained below the fence. Allocation of unmarked fish to treatments is based on the ratio of marked to unmarked fry liberated, ratio of marked to unmarked adults returning to the fence, estimated total of marked fish in the catch, and for the Tsolum River below the weir, on the ratio of marked fish observed above and below the weir. Total estimated fry-to-adult survivals (S) then become:

		Marked fish		Unmarked fish	
Source	Location	No.	S(%)	No.	S (%)
TK	Catch	494	0.40	1,482	1.06
TK	Escapement	311	0.25	933	0.67
TK	Total C and E	805	0.65	2,415	1.73
	From fry	124,800	$Su/Sm = 2.67$	140,000	$Zm/Zu = 1.24$
KK	Catch	410	0.37	48	1.00
KK	Escapement	68	0.06	8	0.17
KK	Total C and E	478	0.44	56	1.17
	From fry	109,700	$Su/Sm = 2.68$	4,800	$Zm/Zu = 1.22$

S = survival rate; Z = instantaneous mortality rate; *u* = unmarked fish; *m* = marked fish.

Results obtained on the TK group are strikingly similar to those otbained in the overlapping 1970–72 hatchery evaluation test on pink salmon of the alternate cycle (cf. Bams 1974). Differential mortality due to marking was again low at $Zm/Zu = 1.24$, which is outside the range established for earlier marking experiments reviewed in Parker et al. (1963).

Discussion

The main results emerging from the experiment concern the survival rates to the adult stage and aspects of migration through inshore waters and within the natal river system.

Marked adult returns demonstrated equal survival in fish of both treatments from emergence to reentry into coastal waters. No estimate can be made for survival of the pure Tsolum stock of the same year-class, but, as shown in the following table, both treatment stocks survived as well as the native TT runs of the alternate year-class during the cycles 1968–70 and 1970–72. These latter runs are comparable with the 1969–71 runs in that the fish were, in part, raised by the same incubation method, received identical marks, etc. (data from Bams 1972, 1974). Annual variations in catch and/or recovery efforts during these 3 yr can be ignored because they were far smaller than normal annual variations in ocean survival recorded elsewhere (e.g. Parker 1962).

Year	Source	Catch as % of fry
1970	Hatchery	0.48
1970	Wild	0.41
1972	Hatchery	0.22
1972	Wild	0.22
1973	TK (hatchery)	0.40
1973	KK (hatchery)	0.37

Seemingly normal survival to the adult stage of fish of the transplanted pure donor stock is undoubtedly a major finding of this experiment. Dozens of within-range pink salmon transplants were attempted during the last six decades but, to my knowledge, none were evaluated for ocean survival prior to fishing mortality. Therefore, most transplants may have failed in not returning significant numbers of adults to the stream of release and/or incubation (cf. Neave 1965), but the possibility exists that returns to the general area were obtained but went unrecognized. This notion is supported by reportedly substantial commercial catches in the Puget Sound area in 1924 of adult pinks believed to have resulted from a release of some 14 million transplanted fry in 1923. The notion is not supported, however, by the much larger fry output of the next cycle, including 30 million transplanted fry, which, evidently, produced a catch of less than 25,000 fish and would certainly indicate a scarcity of returns to the area. In any event, our test clearly demonstrated that a transplanted stock can survive at a normal level to time of entry into coastal waters.

Adult returns to the hatchery stream comprised the main test criterion. It is here that the presumed survival value of locally adapted genes would be expressed in aggregate. It was shown that subsequent to passage through the inshore waters the relative frequencies of occurrence shifted greatly in favor of local genotype fish. At the hatchery weir $\hat{p}_{TK}$ was 0.91, expected p was 0.53, which corresponds to a shift in abundance ratio of 10:1 from 1:1. Because the fish returned to the coast in proportion to size of fry populations as released, it follows that the missing KK fish failed to enter the Tsolum River and presumably went astray between Johnstone Strait and the mouth of the Courtenay River. On the basis of the number of TK fish recovered, 82 KK fish, or 89% of the number expected, were not accounted for at the weir, and an additional 74, or 68% were missing from the fish counted in the Tsolum River below the weir. The total estimated available escapement of KK fish was 273, of which 205 or 75% went astray. Spot checks of adjacent rivers and the donor stream failed to locate any of the missing fish, which was perhaps to be expected on the basis of sample sizes which were small in relation to totals of fish available in various areas. However, even without knowledge of where the fish went, the available information strongly suggests deficiency in homing ability of the pure donor fish. This result demonstrates the serious threat to which neighboring conspecific populations could be exposed in the event of indiscriminate large-scale transplants. In addition to possible introduction of disease, stray fish entering stable populations would contribute to genetic load and alter fitness, with, as the most likely outcome, subsequent depression of survival (cf. Calaprice 1969).

It has been suggested that the TK group could be viewed as the result of immigration of Kakweiken stock into the Tsolum stock and that the results obtained indicated very little loss of fitness in the progeny. Accepting this view would, however, not negate the general argument of expected loss of overall fitness in stable, locally adapted populations by uncontrolled immigration. We applied some criteria in selecting the donor stock, but the choice may have been a fortuitous one in respect of several characteristics we did not knowingly select for. In the face of extensive evidence for genetically controlled differences in many characteristics of adaptive significance to local populations (demes) in other organisms (e.g. Mayr 1966), the general warning concerning genetic pollution associated with indiscriminate transplants is not at all challenged (see a position paper prepared by the Genetics Committee of the Alaska District of the American Institute of Fishery Research Biologists, Anon. 1975). Furthermore, the next paragraph indicates a possibly serious reduction in fitness observed in this particular hybrid population.

It was demonstrated that, relative to the catch, TK fish returned to the river as well as pure TT progeny of the alternate cycle in two earlier operations. Accuracy of homing within the river system, however, differed markedly. Earlier data on numbers of marked hatchery fish spawning above and below the hatchery weir suggested the following proportions of fish passing through the weir: in 1970 — 80% and in 1972 — 86%. The corresponding figures for 1973 were TK fish 45.6%, and KK fish 22.2% (see also Table 4). Hence, degree of within-river homing in all hatchery-raised fish was strongly associated with frequency of inherited local genes: TT, 80–86%; TK, 46%; and KK, 22%. Genetic control of homing ability is therefore implicated not only in locating the home stream, but also the tributary in (on) which the fish were incubated and from which they migrated as fry. We observed the following progression. Environmental "imprinting" alone during incubational and/or migrational stages was sufficient to return some fish of the pure donor stock to the river system and to the native tributary. Addition of the paternal "home" gene complement increased return to the river system to a level comparable to that of the alternate cycle and also increased substantially relative return to the native tributary. However, only when both parental complements were present was a high accuracy of return to the native stream obtained.

Although this supposition may well fit the actual case, an unknown proportion of the Tsolum male brood stock consisted of fish presumably native to areas of the Tsolum River other than Headquarters Creek. Therefore, at least a proportion of the "nonhoming" TK fish may have tended to home to the parental area of the river. Such a trend would diminish the proportion of fish entering the weir and bias downward the influence of the T-genes as estimated by return to the hatchery stream.

The only comparable work I am aware of was two tests carried out by the Washington State Department of Fisheries in 1957 and 1963 on stocks of chinook salmon. The first experiment used donor stock from Green River (Puget Sound area) moved to Grays Harbour (coastal). Treatments consisted of pure donor, hybrids and pure coastal, all raised in and released from the same hatchery. The treatments were somewhat confounded because the coastal males used for hybridization were not native to the hatchery stream, whereas the pure coastals were. Adult returns to the hatchery of 2- to 5-yr-old fish totaled only 52 fish of which 71% were local stock, 21% hybrids and 8% donor. Estimated fishery catches totalled 108 fish comprising 16% local, 84% hybrid, and no donor fish. Indications were that the hybrids returned better than the pure donor, but not as well as the local fish (Anon. 1963). The second experiment was carried out on 1963 Deschutes River (Puget Sound) donor material and Simpson River (coastal) males, but, to my knowledge, no returns have been reported from more than 350,000 marked fry released in the spring of 1964 (Anon. 1965).

Although largely speculative, some interesting propositions may be advanced concerning genetic control of timing of adult returns. To start with, mean times of river entry of Kakweiken and Tsolum stocks differed considerably in the parent year. By virtue of the artificial propagation process spawning dates of the experimental groups were made identical, but means for the total stocks were not. The Tsolum males we used are thought to have been fairly representative of the run as a whole, but the Kakweiken fish were collected after the major part of the run had ascended the river. Observations by Fisheries Service (Operations) personnel put the estimated mean (peak time of river entry) in the 3rd wk of September (Anderson personal communication), i.e. about 10 days earlier than the Tsolum River stock. Even though we obtained our gametes from a late segment of the population, the central tendency of time of return in the pure donor progeny was somewhat closer to that of the total population but considerably earlier than that of the native Tsolum population. These events would be expected if time of return were under genetic control.

As indicated by recorded mean dates the Tsolum male gene complement did not change mean time of adult return. Passage through the fishery (mean recovery dates and variances) also indicated almost identical migration patterns in both treatments. These events indicate a lack of dominant male genetic control. However, mean time of creek entry of both marked populations differed significantly from the unmarked population. As mentioned earlier, we believe, on the basis of observed consistency of time of return in other pink salmon stocks, that these unmarked fish were TT (pure local) stock returning at their normal time. TK fish returning simultaneously with KK but not with TT would be the outcome of dominant maternal control. Adult migration appears controlled by genes differing from those controlling fry migration. The latter showed a difference in timing associated with the male complement, TK differed from KK, but the former did not, both TK and KK fish arriving at the same time.

There is no evidence from earlier marked fish returns (Bams 1972, 1974) that the hatchery treatment alone could create a difference in time of return when compared with naturally propagated fish. Also, timing of Headquarters Creek adult runs, in the even years, varied little, far less than the difference observed here. If this suggestion is generally true, timing of hybrid returns would be that of the donor stock and selection of such a stock would have to be weighed heavily by this criterion in possible future applications.

The discussion on surmised genetic control of timing of the returns is short on data and long on inference. However, because required experimentation, such as evaluation of differences in timing of marked offspring in a four-way cross experiment, is not likely to be carried out in the near future and, further, because improved transplanting techniques are desired now, and are most likely to involve the cross we tested, there is a need to extract even merely suggestive evidence, if by doing so the likelihood of failure of any attempted transplant may be reduced.

Concluding Remarks

The results of this experiment may be related to application in future pink salmon transplants. Assuming our results to be generally applicable, pure donor transplants of selected stocks could produce returning adults as well as a native stock would and, hence, contribute fully to a fishery. Some returns to the natal stream could be expected; however, these would be only a fraction of those produced by a native stock. "Hybrid" transplants, using available residual males, would produce returning adults equally well and, furthermore, provide returns to the home stream as well as local fish would. This feature would be of paramount importance if a self-sustaining stock were desired. Especially if the hatchery facility were located on a small system or near the mouth of a larger system, returns to the site would seem assured. However, in subsequent cycles a temporary drop in production, at least to the site, would be expected for various reasons. Gene segregation in F_2 and later generations would result in the production of genotypes differing from the F_1 hybrids and both more and less fit types would occur (e.g. TT and KK alleles in our experiment). Nonadaptive donor characteristics would be selected against. Until the frequency of such genes in the population is greatly reduced, considerable mortality (including straying) could occur before the stock stabilized at a production level. Therefore, a commitment to follow through for several generations subsequent to the first introduction should be an essential part of any attempt to introduce a continuing stock. Of great biological concern is that proportion of the mortality that occurs in the form of nonhoming of returning adults. Its occurrence and extent was clearly demonstrated in this experiment. Such strays may enter "barren" streams in which case they are most likely to quickly die out, as indicated by the record of past transplants into such streams. It is conceivable they would establish a new run; the effect of this could vary from the beneficial to the disastrous, depending on response and value of the preexisting ecosystem. Strays joining conspecific stocks may extract a high cost by contributing to genetic load and lowering local adaptiveness, which, as a rule, is not beneficial to a stable population (see p. 2723).

The results of this transplant experiment will undoubtedly fire the imagination of some production-oriented people and considerable potential is, indeed, indicated. However, indiscriminate application is likely to do more harm than good and such harm may be irreversible and quite costly. Any attempt at introduction should be preceded by a thorough evaluation of biological aspects affecting any ecosystems with which the new stock (including its strays) would interact.

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ANON. 1963. Washington State Dept. of Fish. Annu. Rep. 72: 88–90.

1965. Washington State Dept. of Fish. Annu. Rep. 74: 106–107.

1975. Salmon hatcheries — moving too fast? Alaska, Seas and Coasts 3: 8–10.

BAMS, R. A. 1972. A quantitative evaluation of survival to the adult stage and other characteristics of pink salmon (*Oncorhynchus gorbuscha*) produced by a revised hatchery method which simulates optimal natural conditions. J. Fish. Res. Board Can. 29: 1151–1167.

1974. Gravel incubators: a second evaluation on pink salmon, *Oncorhynchus gorbuscha*, including adult returns. J. Fish. Res. Board Can. 31: 1379–1385.

1976. Results of a pink salmon transplant using males native to the recipient stream. Fish Mar. Serv. Res. Dev. Tech. Rep. 642: 46 p.

BAMS, R. A., AND D. G. CRABTREE. 1976. A method for pink salmon propagation: the Headquarters Creek experimental hatchery 1968–74. Fish. Mar. Serv. Res. Dev. Tech. Rep. 627: 70 p.

CALAPRICE, J. R. 1969. Production and genetic factors in managed salmonid populations, p. 377–388. *In* T. G. Northcotte [ed.] Symposium on salmon and trout in streams. H. R. MacMillan Lectures in Fisheries, Univ. British Columbia, Vancouver, B.C.

MAYR, E. 1966. Animal species and evolution. Harvard University Press, Cambridge, Mass. 797 p.

NEAVE, F. 1965. Transplants of pink salmon. Fish. Res. Board Can. MS Rep. 830: 37 p.

PARKER, R. R. 1962. A concept of the dynamics of pink salmon populations, p. 203–211. *In* N. J. Wilimovsky [ed.] Symposium on pink salmon 1960. H. R. MacMillan Lectures in Fisheries, Univ. British Columbia, Vancouver, B.C.

1964. Estimation of sea mortality rates for the 1960 brood-year pink salmon of Hook Nose Creek, B.C. J. Fish. Res. Board Can. 21: 1019–1034.

PARKER, R. R., E. C. BLACK, AND P. A. LARKIN. 1963. Some aspects of fish marking mortality. Int. Comm. Northwest Atl. Fish. Spec. Bull. 4: 117–122.

SIEGEL, S. 1956. Non-parametric statistics for the behavioral sciences. McGraw-Hill, Inc., New York, N.Y. 312 p.

SOKOL, R. S., AND F. J. ROHLF. 1969. Biometry, the principles and practice of statistics in biological research. W. H. Freeman and Co., San Francisco, Calif. 776 p.

STEEL, R. G. D., AND J. H. TORRIE. 1960. Principles and procedures of statistics. McGraw-Hill, Inc., New York, N.Y. 481 p.

Long-Term Effects of Methylmercuric Chloride on Three Generations of Brook Trout (*Salvelinus fontinalis*): Toxicity, Accumulation, Distribution, and Elimination

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McKim, J. M., G. F. Olson, G. W. Holcombe, and E. P. Hunt. 1976. Long-term effects of methylmercuric chloride on three generations of brook trout (*Salvelinus fontinalis*): toxicity, accumulation, distribution, and elimination. J. Fish. Res. Board Can. 33: 2726–2739.

During a 144-wk period three generations of brook trout (*Salvelinus fontinalis*) were continuously exposed to mean water concentrations of methylmercuric chloride (MMC) of 2.93, 0.93, 0.29, 0.09, 0.03, and <0.010 (control) μg Hg/liter. During the first 39 wk the highest concentration (2.93 μg Hg/liter) produced deformities and 88% mortality of first-generation adults. At an MMC concentration of 0.93 μg Hg/liter, second-generation trout developed deformities and all but one female died during the 108-wk exposure. No significant effects on survival, growth, or reproduction of second-generation trout were noted at any of the lower MMC concentrations. No toxic symptoms were observed in the third generation at the three lowest MMC concentrations. The maximum acceptable toxicant concentration (MATC) for brook trout exposed to MMC in this water with a hardness of 45 mg/liter (as CaCO$_3$) and a pH of 7.5 fell between 0.93 and 0.29 μg Hg/liter. The mean 96-h LC50 for yearling (200 g) and 20-wk-old (12 g) juvenile brook trout exposed to MMC was 75.0 μg Hg/liter, and the application factor (MATC/96-h LC50) lies between 0.004 and 0.013. The accumulation rate of mercury by eight selected tissues of first-generation trout exposed to MMC was relatively rapid at all MMC concentrations tested. The 2-wk tissue Hg: water Hg concentration factors ranged from 1×10^3 to 12×10^3, depending on the tissue, whereas after 28–38 wk of exposure the maximum tissue Hg: water Hg concentration factors for both first- and second-generation trout ranged from 6.9×10^3 to 6.3×10^4. The blood, spleen, and kidney accumulated mercury most rapidly and contained the highest residues in both first- and second-generation trout followed by liver, gill, brain, gonad, and muscle in order of decreasing mercury residues. There was no significant elimination of mercury from the tissue of first- or second-generation fish, yet a "steady state" (micrograms mercury per gram = constant) was reached in all tissues after 20–28 wk of continuous water exposure. Monomethylmercury made up 90–95% of the total mercury present in muscle, the only tissue analyzed for this compound. Mean muscle residues in first-generation trout, dying after 16–28 wk of exposure to 2.93 μg Hg/liter and in second-generation trout, dying after 64–100 wk of exposure to 0.93 μg Hg/liter, were 23.5 and 9.5 μg Hg/g, respectively.

McKim, J. M., G. F. Olson, G. W. Holcombe, and E. P. Hunt. 1976. Long-term effects of methylmercuric chloride on three generations of brook trout (*Salvelinus fontinalis*): toxicity, accumulation, distribution, and elimination. J. Fish. Res. Board Can. 33: 2726–2739.

Trois générations d'ombles de fontaine (*Salvelinus fontinalis*) ont été exposées de façon continue durant une période de 144 semaines à des concentrations moyennes dans l'eau de chlorure de méthyl-mercure (CMM) de 2.93, 0.93, 0.29, 0.09, 0.03 et <0.010 (témoin) μg Hg/litre. Au cours des 39 premières semaines, la concentration maximale (2.93 μg Hg/litre) produit des difformités et des mortalités de 88% des adultes de première génération. A une concentration de CMM de 0.93 μg Hg/litre, les ombles de deuxième génération souffrent de difformités et tous, sauf une femelle, meurent pendant l'exposition de 108 semaines. Nous n'avons pas observé d'effets significatifs sur la survie, la croissance ou la reproduction d'ombles de deuxième génération à aucune des concentrations plus faibles de CMM. La troisième génération ne montre aucun symptôme toxique aux trois plus basses concentrations de CMM. La concentration maximale acceptable de toxique (CMAT) pour des ombles de fontaine exposés au CMM dans cette eau d'une dureté de 45 mg/litre (comme CaCO$_3$) et un pH de 7.5 se situe entre 0.93 et 0.29 μg Hg/litre. La CL50 moyenne après 96 h pour des poissons de l'année (200 g) et des jeunes âgés de 20

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semaines (12 g) exposés au CMM est de 75.0 μg Hg/litre, et le facteur d'application (CMAT/CL50 après 96 h) se situe entre 0.004 et 0.013. Le taux d'accumulation du mercure par huit tissus choisis d'omble de fontaine de première génération exposé au CMM est relativement rapide à toutes les concentrations de CMM essayées. Les facteurs de concentration Hg du tissu : Hg de l'eau après 2 semaines varient de 1×10^3 à 12×10^3, selon le tissu, alors qu'après 28–38 semaines d'exposition, les facteurs maximums de concentration Hg du tissu : Hg de l'eau, tant pour les ombles de première de deuxième génération, varient de 6.9×10^3 à 6.3×10^4. Le sang, la rate et le rein accumulent le mercure le plus rapidement et contiennent les résidus les plus élevés, tant chez les ombles de première que de deuxième génération. Viennent ensuite le foie, les branchies, le cerveau, les gonades et le muscle, par ordre décroissant de résidus de mercure. Il n'y a pas élimination significative de mercure du tissu de poissons de première et de deuxième génération, mais on constate un « état d'équilibre » (microgrammes de mercure par gramme = constante) dans tous les tissus après 20–28 semaines d'exposition continue. Le monométhylmercure constitue jusqu'à 90–95% du mercure total présent dans le muscle, le seul tissu dans lequel nous avons dosé ce composé. Les résidus moyens dans le muscle d'ombles de première génération, morts après 16–28 semaines d'exposition à 2.93 μg Hg/litre, et dans des ombles de deuxième génération, morts après 64–100 semaines d'exposition à 0.93 μg Hg/litre, sont de 23.5 et de 9.5 μg Hg/g respectivement.

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THIS study was designed to determine the acute and chronic toxicity (involving survival, growth, and reproduction) of low concentrations of methylmercuric chloride (MMC) in water to three generations of brook trout (*Salvelinus fontinalis*) exposed over a 144-wk period, and to ascertain from the fish tissues the accumulation rate, distribution, and elimination rate of mercury. In addition, the mercury residues in trout killed by mercury during the exposure were investigated to determine tissue mercury concentrations that would be expected to cause symptoms of toxicity in brook trout.

Little information exists on concentrations of methylmercury in water that are chronically toxic to fish or that cause unacceptable residues except for the work of the following researchers: Matida et al. (1971), who exposed rainbow trout to low concentrations of MMC in both water and food and observed toxic symptoms; Hannerz (1968) who studied the accumulation and distribution of several forms of mercury, including methylmercury, in aquatic animals both in the laboratory and under field conditions; Burrows and Krenkel (1973) who observed the uptake, distribution, and half-life of methylmercury in bluegills exposed to low water concentrations for 5 days; and Olson et al. (1975), who exposed fathead minnows to low water concentrations of MMC and found no effects on survival at concentrations causing unacceptable residues. No previous studies are available on the chronic toxicity of low water concentrations of MMC to fish involving several generations and all developmental stages in the life cycle. The present study allows a comparison of first-, second-, and third-generation effects, which are of special concern when dealing with toxicants that have a high bioaccumulation potential.

Materials and Methods

PHYSICAL AND CHEMICAL

Exposure system — The exposure system consisted of a proportional diluter (Mount and Brungs 1967) that delivered 2 liters of test water per cycle to flow-splitting chambers (Benoit and Puglisi 1973). These chambers divided each of the six diluter concentrations four ways into two duplicate adult spawning tanks and two duplicate larval growth tanks. Larval growth tanks were located above and drained into the adult spawning tanks containing the same concentration of toxicant. Flow rates of test water averaged 790 ml/min for the adult spawning tanks and 120 ml/min for the larval growth tanks. The replacement time for 90% of the test water was calculated (Sprague 1969) to be approximately 4 h in both the adult spawning tanks and in each larval growth chamber. The adult spawning containers were 91.4 × 30.5 × 38.0-cm high stainless steel tanks with a 30.5-cm standpipe. Larval rearing facilities consisted of an outer glass tank, 55.0 × 27.5 × 15.0 cm, with an inside tank, 42.5 × 25.0 × 15.0 cm, divided lengthwise into two 12.5-cm wide growth chambers. Water depth in each chamber measured 12.7 cm. Gentle aeration was used in all tanks throughout the exposure. Debris was siphoned from all tanks Monday through Friday, and all tanks were scrubbed once a week. All adult spawning tanks were covered with fiberglass mesh screens and were concealed by curtains to minimize disturbance of fish. Embryos and alevins up to 4 wk old were protected from direct light by black plastic covers in nearly complete darkness (0.02 lm). Fluorescent lamps were used in combination as the main source of illumination. They were used in conjunction with incandescent bulbs wired to a light timer, which controlled the photoperiod, and an automatic dimmer, which simulated

TABLE 1. Survival, spawning, and egg production of first- and second-generation brook trout (*Salvelinus fontinalis*) exposed to various concentrations of methylmercuric chloride.

	Total mercury (μg/liter)											
	2.90[a] ± 0.122	2.97 ± 0.148	0.91 ± 0.026	0.96 ± 0.030	0.29 ± 0.020	0.29 ± 0.020	0.09 ± 0.020	0.09 ± 0.008	0.03 ± 0.002	0.03 ± 0.002	<0.01 (Control)	
Item	A[b]	B	A	B	A	B	A	B	A	B	A	B
First generation												
Adult survival during spawning (%) (Sept. 1971–Feb. 1972)	0	25	100	100	100	100	100	75[c]	100	100	100	75[c]
No. mature males	1	1	2	2	1	2	2	2	2	2	2	2
No. mature females	2	3	2	2	2	2	2	2	2	2	2	2
No. females spawning	0[d]	0	2	2	0	2	2	2	2	2	2	2
Mean spawnings per female	0	0	3	2.5	0	1.5	1	3	3	3	2	3.5
Mean viable eggs spawned per female	0	0	648	89	0	382	245	615	358	240	539	472
Second generation												
Adult survival during spawning (%) (Dec. 1973–Feb. 1974)	0[e]	0[e]	16	0[e]	0[e]	83	66	100	83	83	100	83
No. mature males	0	0	2	0	2[f]	2	2	2	2	2	2	2
No. mature females	0	0	4	0	3[f]	4	4	4	4	4	4	4
No. females spawning	0	0	0	0	3	2	2.5	2	2	2	2.5	0.5
Mean spawnings per female	0	0	0	0	2.7	4	3.7	3	4	1.5	3	1
Mean viable eggs spawned per female	0	0	0	0	296	444	0[g]	430	522	421	609	446

[a] Mean and 95% confidence interval; [b] A and B represent duplicate adult spawning tanks; [c] Disease-related deaths; [d] All spawners died before or during spawning; [e] No successful reproduction occurred in first-generation trout; therefore, there were no second-generation spawners; [f] Spawners transferred from duplicate B; [g] All fish badly diseased (no viable eggs produced).

sunrise and sunset (Drummond and Dawson 1970). Maximum light intensity measured 8.5 lm at the water surface in the adult spawning tanks.

Water characteristics — Test water throughout the exposure was obtained directly from Lake Superior. It was passed through an ultraviolet sterilizer to kill possible pathogens and was heated to the proper temperature by a hot water heat-exchange system. Throughout the juvenile–adult exposures, seasonal temperatures ranged from 9 to 15 C. Temperatures were maintained at 9 ± 1 C during all spawning, egg-incubation, and 12-wk alevin–juvenile growth studies. Total hardness, alkalinity, acidity, and pH determinations were made on all tanks, alternately, one tank a week (mean total hardness = 45 mg/liter as $CaCO_3$, range 45–46; mean acidity = 3.9 mg/liter as $CaCO_3$, range 1.9–6.1; mean alkalinity = 42 mg/liter as $CaCO_3$, range 41–44; pH = 6.9–7.6). Dissolved oxygen was measured in a different tank twice a week (mean dissolved oxygen = 7.7 mg/liter, range 4.3–12.1). Chemical measurements were made according to standard methods (APHA et al. 1971).

Toxicant solution — Nineteen liters of MMC (expressed as micrograms Hg per liter) stock solution were prepared each week and introduced from a Mariotte bottle to the diluter by a chemical-metering apparatus (McAllister et al. 1972). During the 144-wk exposure, mean water concentrations of MMC were 2.93, 0.93, 0.29, 0.09, 0.03, and <0.01 (control) μg Hg/liter. Once a week a water sample (150 ml) from one duplicate tank at each mercury concentration was analyzed for total mercury (Table 1). Total mercury in the test water was analyzed by flameless atomic absorption (Kopp et al. 1972; Olson et al. 1975). Accuracy for total mercury in water was checked by the method of known additions by adding 0.10 and 1.0 μg Hg/liter as MMC to control water and determining the percentage recovery. Water samples for recovery estimates and reagent blanks were analyzed each time samples from the test system were analyzed, and the resulting mean recovery and 95% confidence interval for the entire exposure period was 101 ± 0.18%. Precision was determined by addition of MMC at 0.10 and 1.0 μg Hg/liter to distilled water and analyzed in triplicate. This was repeated 10 times, and the results were within 2% of each other. Additional chemical tests, carried out according to the methods of Olson et al. (1975), showed that 90–92% of the mercury in the test tanks was organic. Thus little, if any, demethylation occurred in this system.

BIOLOGICAL

This study involved 1) exposure of yearling brook trout through spawning (May 1971–February 1972); 2) exposure of the offspring from the initial parental group through spawning (November 1971–February 1974); and 3) exposure of offspring from the second parental group to the early juvenile stage (November 1973–May 1974) (Fig. 1). Biological methods for these three generations will be given separately.

First generation — Yearlings through adult spawning — Yearling brook trout (50–70 g) were obtained from the Cedar Island Hatchery in Brule, Wisconsin. The fish were acclimated to laboratory conditions for 4 wk before starting the exposure. The experiment began in May 1971 (Fig. 1), when 32 fish were placed by stratified random assignment into each of the 12 duplicate exposure tanks.

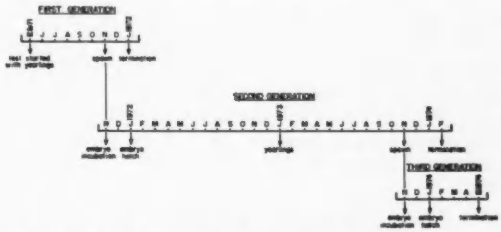

FIG. 1. Chronology of three generations of mercury-exposed brook trout (*Salvelinus fontinalis*).

Prior to spawning, fish were removed from the system after 2 and 8 wk and eight tissues (muscle, liver, kidney, spleen, brain, gonad, gill, and blood) from 4–10 trout from each mercury concentration were taken for mercury-residue determinations.

As the spawning season approached in September 1971, the remaining fish in each tank were separated into groups of males and females. From these groups fish were chosen randomly to obtain a 1:1 sex ratio consisting of two males and two females per duplicate tank. All remaining fish (4–10) in each duplicate were killed for residue analyses (same eight tissues) at 20 wk and one spawning substrate was placed in each tank. Spawning commenced in mid-November and continued into mid-December. Experimental procedures used during spawning and parental termination were the same as those described by McKim and Benoit (1971). After spawning ceased (39 wk) all remaining fish were killed for residue analysis (same eight tissues) except for two fish at each MMC concentration. These were then removed from the exposure tanks and placed in control water for 16 wk. At the end of the 16-wk period the same eight tissues, plus the remainder of the carcass, were analyzed for mercury. This allowed an estimate of the rate of loss of mercury from the tissues. Tissue samples (except for blood) were also taken from the fish that died during the 39-wk test.

All tissues were individually wrapped in cellophane, placed in sealed plastic bags, and stored at −20 C before analysis. All mercury tissues residues were determined by flameless atomic absorption (Olson et al. 1975). The reported values are based on wet weight. Accuracy for total mercury in fish tissue was checked by addition of MMC to a duplicate sample from the same homogenate. These recovery samples were analyzed with each group of tissues; usually 0.05 or 0.1 μg of MMC was added, and an overall mean recovery of 97.7% was obtained.

Second generation — Embryo through adult spawning — Exposure of the second generation of brook trout to MMC through one complete life cycle began with the spawning of the first-generation fish (Fig. 1). Biological methods used during the second-generation incubation of embryos and survival and growth studies of alevins and juveniles were the same as those described by McKim and Benoit (1974). Two hundred embryos were also transferred (as above) from the control tanks to each methylmercury concentration and fifty embryos from the two highest methylmercury concentrations to the control tank. Exposure of these transferred embryos ended 4 wk after hatching. Weights were determined on all alevins at hatch and on a subsample of 40 juvenile fish per concentration 16 wk after hatch. Second-generation juveniles were transferred from the larval growth chambers to the adult spawning tanks 12 wk after hatching.

During September 1973 second-generation fish were randomly thinned to a sex ratio of two males to four females in all tanks. Two artificial laboratory spawning substrates (Benoit 1974) were then placed in all tanks, and spawning commenced in mid-November 1973. Experimental methods used during spawning and parental termination were the same as those described for first-generation fish.

Embryos, alevins, and juveniles from all mercury concentrations and controls were sampled and preserved both by freezing and in 10% buffered formalin for later whole-body mercury-residue analysis. No detectable differences in mercury residues were measured in any tissues preserved by freezing or 10% formalin. Samples were taken at spawning, at hatch, and at 8, 20, and 28 wk after spawning. Two to six second-generation fish from each duplicate tank were also randomly selected for residue analyses at ages 40, 52, 76, 88, and 108 wk. Blood was drawn, and gill, liver, kidney, gonad, spleen, muscle, and brain tissues were dissected from these fish. After 108 wk two fish remained at each mercury concentration and were transferred to control water to determine a 12-wk dissipation rate. All blood, tissue, and remaining carcass samples from second-generation fish, including the tissues used to determine a dissipation rate, were analyzed for total mercury. The same procedures described for first-generation fish were used. In addition to the total mercury measurements made on second-generation trout muscle, the amount of methylmercury present was determined on 12 separate samples by methods described by Longbottom et al. (1973) and Talmi and Crossman (1973).

Third generation — Embryo through 12-wk-old alevins — The third-generation mercury exposure began with the spawning of the second-generation fish (Fig. 1). This generation was exposed throughout the embryo stage and for 12 wk following hatch. Biological methods were the same as those given for the second-generation alevins, except that weights of all surviving juvenile fish 12 wk after hatch were determined.

Trout diets and treatment of diseases and ectoparasites — All fish were fed measured amounts of a commercial trout food (Oregon Moist, Glencoe Mills, Silver Cup, and Ewos)[1] daily. Food rations were calculated bimonthly by multiplying the total body weight of each individual group of exposed fish by a percentage of body weight ranging from 0.7 to 3.4% depending on the average weight of the fish and water temperature.

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[1] Selection of these commercial diets was based on pesticide content and availability.

Diseases and ectoparasites were treated on several occasions throughout the exposure with neomycin, tetracycline, and formalin as recommended by The Committee on Methods for Toxicity Tests with Aquatic Organisms (1975). Secondary fungal growth (*Saprolegnia*) was controlled by swabbing the infected area with a 1% solution of malachite green.

Acute toxicity tests — Two 96-h acute toxicity tests were conducted at 12 ± 1 C with yearlings (102 g) from the same group of trout used to begin the chronic test and with 5-mo-old (12-g) juvenile brook trout from another group. These continuous-flow tests with 10 fish per duplicate were conducted according to methods described by The Committee on Methods for Toxicity Tests with Aquatic Organisms (1975).

Data handling and statistical analyses — The estimated total weight of young produced per female is the product of eggs per female, hatchability, alevin–juvenile survival, and juvenile weight. The ratio of the mean estimated total weight of young per female between fish exposed to methylmercuric chloride and controls allows a comparison of second- and third-generation results and provides one number that represents all of the observations recorded in a brook trout chronic toxicity study in relation to the controls. Spawning data, half-life data, adult and juvenile weights, and the estimated total weight of young per female were subjected to an analysis of variance with an *F* test of significance ($P = 0.05$) (Steel and Torrie 1960).

Results

TOXICITY

First generation — During the 39-wk first-generation brook trout study, 88% of the trout exposed to MMC at a concentration of 2.93 μg Hg/liter (mean of duplicate A and B) died before or during the spawning period, and no spawning took place (Table 1). Development and behavior of first-generation trout exposed to MMC was also affected at the high (2.93 μg Hg/liter) concentration, where loss of appetite, muscle spasms, and trunk deformities appeared after 20 wk of exposure.

Spawning behavior, as evidenced by the number of spawnings produced per female, survival and growth were not affected at the lower MMC concentrations of 0.03 to 0.93 μg Hg/liter (means of duplicate A and B) (Table 1). The means and 95% confidence intervals of the lengths and weights of all test fish at spawning were 25.0 ± 1.9 cm and 187.6 ± 39.8 g, respectively. The number of viable eggs produced per female was not significantly reduced ($P = 0.05$) at any of the MMC concentrations where spawning occurred. At 0.93 μg Hg/liter (mean of duplicate A and B) the number of viable eggs produced was markedly reduced in duplicate B (Table 1), but duplicate A produced more viable eggs per female than any of the lower MMC concentrations.

Second generation — No adverse effects were seen on hatchability or on growth or survival of second-generation trout except for decreases ($P = 0.05$) in hatchability and juvenile weight at 0.93 μg Hg/liter (Table 2). When we considered the product of eggs per female, hatchability, alevin–juvenile survival, and juvenile weight as a group, and the products (estimated total weight of young per female) of each duplicate were compared, no significant differences could be shown ($P = 0.05$) at any of the four lower MMC concentrations (0.03–0.93 μg Hg/liter). However, the low ratio of the mean estimated total weight of young per female indicated a possible effect at 0.93 μg Hg/liter (Table 2).

No effects were noted on second-generation embryos transferred from the control tank at spawning to all of the MMC exposure tanks for up to 4 wk after hatching, except at the highest MMC concentration (2.93 μg Hg/liter), which resulted in no development beyond the neural keel and death of all the embryos.

Results for survival and reproduction of second-generation trout exposed to MMC throughout one complete life cycle are also presented in Table 1. No second-generation fish were produced in the 0.96 μg Hg/liter tank of the duplicate 0.93 μg Hg/liter system, so that no second-generation fish were available for spawning (Table 1). Those second-generation fish surviving at 0.91 μg Hg/liter in the other tank of the 0.93 μg Hg/liter duplicate system were all deformed in the brancheostegal–opercular region, and all but one female died during spawning. The behavior of these trout was abnormal before death; body movements were sluggish and respiratory movements were rapid. These effects during spawning at 0.93 μg Hg/liter support the earlier observations on the low ratio of the mean estimated total weight of young produced per female. The three lower MMC concentrations did not significantly affect the number of viable eggs spawned per female ($P = 0.05$), or the survival or growth of second-generation trout (Table 1). The means and 95% confidence intervals of the lengths and weights of all test fish at spawning were 28.3 ± 1.9 cm and 304 ± 50.5 g, respectively.

TABLE 2. Estimated total weight of second-generation (16-wk-old) and third-generation (12-wk-old) brook trout produced per female at various methylmercuric chloride concentrations.

Mean total mercury concn (μg/liter)	Duplicates	Estimated[a] eggs/female ×	Hatchability (%) ×	Alevin–juvenile survival (%)[b] ×	Individual juvenile weight (g) =	Estimated total wt of young/female (g)	Ratio of mean estimated total wt of young/female (exposed : control)
				Second generation			
2.93	A	(No spawning)	—	—	—	—	—
	B						
0.93	A	583	84 (6)[c]	90	2.20[d]–0.15[e] (20)[f]	969.7	0.32
	B	80	0 (3)	0	0	0	
0.29	A	0[g]	—	—	—	—	0.77
	B	344	98 (3)	98	3.58 ± 0.08 (20)	1182.8	
0.09	A	221	100 (2)	98	2.79 ± 0.21 (20)	604.3	0.69
	B	554	99 (6)	96	2.85 ± 0.19 (20)	1500.6	
0.03	A	322	80 (5)	93	3.33 ± 0.13 (20)	797.8	0.45
	B	216	94 (2)	94	2.99 ± 0.16 (20)	563.0	
< 0.01 (Control)	A	485	97 (4)	98	3.31 ± 0.57 (20)	1526.1	1.00
	B	425	97 (5)	94	3.96 ± 0.24 (20)	1534.6	
				Third generation			
2.93	A	(No spawning)	—	—	—	—	—
	B						
0.93	A	(No spawning)	—	—	—	—	—
	B						
0.29	A	158	61 (4)	80	0.94 ± 0.10 (35)	72.48	0.58
	B	236	94 (2)	98	0.96 ± 0.10 (41)	208.70	
0.09	A	—[h]	—	—	—	—	0.60
	B	229	88 (3)	90	0.81 ± 0.13 (39)	146.91	
0.03	A	278	56 (4)	85	1.18 ± 0.05 (33)	156.15	0.86
	B	224	84 (2)	100	1.40 ± 0.07 (41)	263.42	
< 0.01 (Control)	A	324	71 (6)	98	0.95 ± 0.08 (44)	214.17	1.00
	B	237	87 (2)	96	1.38 ± 0.09 (40)	273.16	

[a] Number of viable eggs produced per spawning female × the probability of spawning (i.e. total spawners/total females = 0.90 for second generation and 0.5323 for third generation); [b] Fifty alevins exposed in each duplicate; [c] Number of spawnings checked for hatchability; [d] Significantly less than control ($P = 0.05$, analysis of variance with the *t*-test); [e] Mean and 95% confidence interval; [f] Number of fish sampled; [g] No eggs spawned in this duplicate; [h] Disease problem.

Third generation — Young third-generation trout were not significantly ($P = 0.05$) affected at the three lower MMC concentrations during the 12 wk after hatching (Table 2). The estimated total weights of 12-wk-old young per female were similar in most cases, although the ratios of the mean estimated total weights of 12-wk-old young per female were less than the control at all MMC concentrations tested.

Maximum acceptable toxicant concentration and application factor — These results establish the "no-effect level" or maximum acceptable toxicant concentration (MATC, as described by Mount and Stephan 1967) for brook trout, in water with a hardness of 45 mg/liter (as $CaCO_3$) and a pH of 7.5, between 0.93 and 0.29 μg Hg/liter. The 96-h LC50s of two continuous-flow bioassays with MMC at 12 ± 1 C for yearling (102-g) and 20-wk-old (12-g) juvenile brook trout were estimated by graphical interpolation to be 65 and 84 μg Hg/liter, respectively (mean = 75.0 μg Hg/liter). Therefore, the application factor (MATC/96-h LC50) lies between 0.013 and 0.004.

RESIDUES

First generation — Mercury accumulated rapidly in all tissues examined in first-generation brook trout (Fig. 2). After only 2 wk of exposure, tissue:water concentration factors were 1×10^3 to 12×10^3 depending on the tissue analyzed, and after 39 wk they ranged from 9×10^3 to 61×10^3.

Even the lowest MMC concentration tested (0.03 μg Hg/liter) produced a significant increase in mercury residues in all tissues analyzed (Fig. 2). The blood, spleen, and kidney accumulated the greatest quantities of mercury. The liver, gill, and brain contained the second greatest amount, and the gonads and muscle had the lowest concentration of mercury. The plasma portion of the blood contained very little mercury (3% of the total) in comparison to the high concentration in the cellular portion (97% of the total). The mercury distribution between the plasma and the red cells remained constant regardless of the mercury levels in the blood or the length of the exposure. The increase of mercury residues in gonads (male and female) after 20 wk was

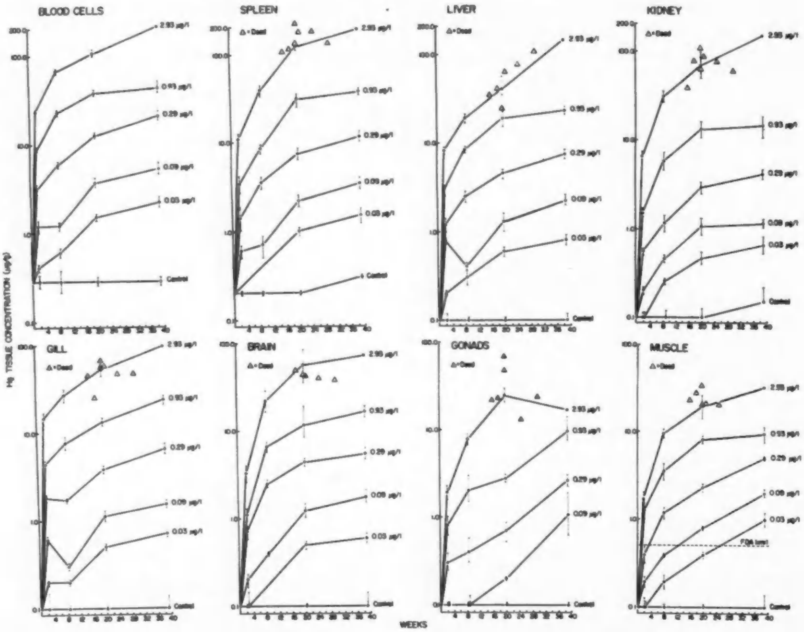

FIG. 2. Mercury concentration (micrograms Hg per gram wet weight) accumulated in selected tissues of living and dead brook trout exposed to various MMC water concentrations over a 39-wk period. Each point is the mean and standard deviation of samples from 4–10 living fish; each triangle represents a single dead fish at the highest mean MMC concentration tested (2.93 µg Hg/liter).

caused by increases in the size of these organs before spawning. The 20-wk sample was taken from "ripe" fish with well-developed large gonads, whereas the 39-wk sample was taken after spawning from fish with small gonads in various stages of regression.

Some individuals showed more resistance to toxication and as a result had higher mercury residues in certain tissues at death (Fig. 2). Brain tissue, however, varied little in the concentration of mercury present at death regardless of time of death. The mean mercury residues (micrograms Hg per gram) in each tissue at death were: spleen, 155.3; liver, 57.6; kidney, 146.6; gill, 47.7; brain, 41.6; gonad, 31.9; and muscle, 23.5.

The mercury concentration in each tissue was directly dependent on the concentration of mercury in the water (Fig. 2). Each MMC concentration in the water was approximately one-third of the next higher concentration and the concentration of mercury accumulated in tissue was also approximately one-third that found at the next higher water concentration. The concentration of mercury in the tissues tended toward a steady state with time at all MMC concentrations. However, there was no decrease in the total mercury taken up. For example, the total mercury in whole fillets of brook trout was determined and plotted in Fig. 3. This indicated that during the 39-wk exposure period there was no apparent reduction in accumulation at any water MMC concentration; in fact, the rate of uptake increased throughout the exposure.

When some of the trout were removed from the MMC exposure and placed in control water for 16 wk, the blood was the only tissue analyzed that lost a significant amount of mercury ($P = 0.05$) (Table 3). Conversely, the kidney showed a significant increase ($P = 0.05$) in total micrograms of mercury at the end of the 16-wk period. The carcass (which included all other tissues) contained 62% of the total mercury body burden; muscle, blood cells, kidney, liver, and gill (in order of decreasing body burden) made up the majority of the rest.

In contrast to the data in Table 3, the micrograms of mercury per gram of tissue dropped sharply after 16 wk in control water (Table 4). The decrease was probably caused by a growth

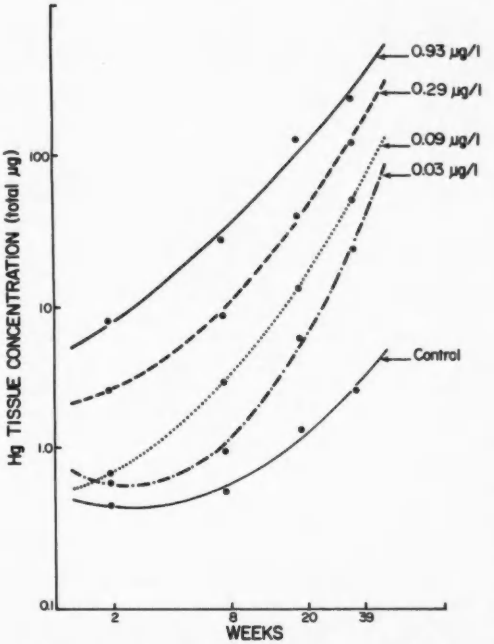

FIG. 3. Total mercury (micrograms Hg wet weight) accumulated in whole fillets of first-generation brook trout exposed to various water concentrations of MMC over a 39-wk period. Each point is the mean of four to five samples.

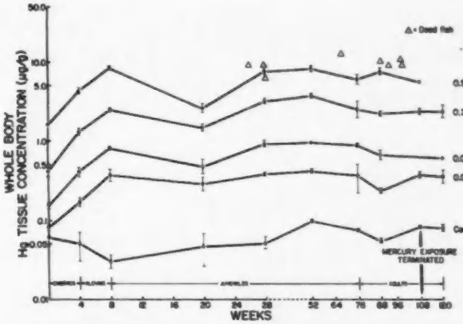

FIG. 4. Whole body mercury concentrations (micrograms Hg per gram wet weight) in living and MMC-killed second-generation brook trout exposed from embryo through adult to various water concentrations of MMC (2.93–0.03 µg Hg/liter). Δ, trout killed by MMC, for the curves representing residues in living trout each point (●) represents the mean and standard deviation of samples analyzed. Sample sizes analyzed were 6–50 for embryo through alevin and 1–6 for juvenile through adult.

"dilution," since the trout had increased their weight 30–45% over the 16-wk period.

Mean bioconcentration factors for a given tissue were approximately the same for all water MMC concentrations except for muscle, which showed a trend toward higher concentration factors at the lower water concentrations (Table 4).

Second and third generation — The second-generation study began with eggs from exposed first-generation parents. A considerable amount of mercury was passed from the parents to the eggs, as seen in Fig. 4 at the beginning of the embryo exposure. A separate set of residue data for third-generation trout was also analyzed, and the amount and pattern of mercury accumulation was essentially the same as in second-generation trout over the first 16 wk of exposure. Analyses of the yolk and embryos separately at hatch indicated approximately 50% of the initial mercury was contained in the yolk. The residue data for second-generation embryos at 2.93 µg Hg/liter and for third-generation embryos at 0.93 µg Hg/liter (neither shown in Fig. 4) were for embryos from stripped eggs, since no spawning occurred at these concentrations. Residues in stripped second-generation embryos at water concentrations of 2.93 µg Hg/liter and stripped third-generation embryos at 0.93 µg/liter were 12.5 ± 0.4 (mean ± SD) and 2.2 ± 0.4 µg Hg/g, respectively. All second-generation embryos were badly deformed at 2.93 µg Hg/liter and did not hatch; third-generation embryos were also deformed, and none survived more than 3 wk beyond hatching at 0.93 µg Hg/liter.

The uptake of mercury was rapid throughout the embryo–alevin stages of development at all MMC concentrations tested (Fig. 4). At the end of the alevin stage when the animals became "free-feeding," the rate of accumulation decreased between 8 and 20 wk, while accumulation rate increased between 20 and 28 wk. After 28 wk the whole-body mercury levels of second-generation brook trout had reached a steady state, and no large fluctuations were noted throughout the remainder of the exposure.

No mercury was eliminated (Fig. 4) from exposed trout 12 wk after MMC treatment ceased. The mean weight of these fish changed very little during the 12-wk period; therefore half-life values could be plotted on a microgram-per-gram basis.

During the full life-cycle second-generation exposure, toxic symptoms and deaths caused by MMC occurred only at 0.93 µg/liter and whole-body mercury residues for these dead fish were plotted in Fig. 4. The mean whole-body mercury residue for these dead fish was 9.5 µg Hg/g, which was essentially the same as residues estab-

TABLE 3. Total mercury (micrograms wet weight) in whole tissues of brook trout: A, exposed for 39 wk to various water concentrations of mercury; B, exposed for 39 wk to various water concentrations of mercury followed by 16 wk in control water. *Mean ±SE; **number of samples in parentheses; ***not measured; assumed to be approximately same as B as seen for total body burden.

	Hg water concn ($\mu g/liter$)					
Tissue	0.93	0.29	0.09	0.03	<0.01 (Control)	Mean % of total body burden
Brain						
A	1.4±0.20* (3)**	1.0±0.04 (3)	0.4±0.003 (3)	0.1±0.04 (3)	0.02±0.01 (3)	0.08±0.02
B	2.3±0.20 (3)	0.9±0.04 (2)	0.3±0.05 (2)	0.1±0.02 (2)	0.03±0.0 (2)	
Blood cells						
A	385.6±62.74 (3)	195.8±20.90 (3)	46.5±6.29 (3)	21.7±5.41 (3)	3.3±0.49 (3)	9.8±1.5
B	252.3±41.77 (3)	111.2±5.15 (2)	34.2±3.70 (2)	19.8±3.20 (2)	3.0±0.04 (2)	
Gill						
A	43.5±7.89 (3)	17.1±0.97 (3)	5.5±0.93 (3)	1.8±0.45 (3)	0.3±0.09 (3)	1.0±0.5
B	46.7±6.35 (3)	7.0±0.06 (2)	2.5±0.02 (2)	1.3±0.28 (2)	0.4±0.12 (2)	
Gonad						
A	4.5±1.31 (3)	3.0±0.50 (3)	0.8±0.07 (3)	0.2±0.03 (3)	0.04±0.0 (3)	0.5±0.1
B	14.1±2.84 (3)	5.5±1.25 (2)	1.5±0.12 (2)	0.8±0.20 (2)	0.1±0.005 (2)	
Kidney						
A	36.5±6.51 (3)	17.4±1.90 (3)	3.4±0.28 (3)	1.9±0.10 (3)	0.3±0.08 (3)	2.0±0.4
B	56.9±8.13 (3)	18.2±3.15 (2)	5.1±0.19 (2)	4.0±0.66 (2)	1.0±0.10 (2)	
Liver						
A	76.3±3.25 (3)	48.1±3.89 (3)	12.5±0.89 (3)	4.5±0.11 (3)	0.6±0.07 (3)	3.0±0.6
B	88.8±19.53 (3)	33.3±4.40 (2)	9.1±1.02 (2)	4.8±0.30 (2)	1.0±0.02 (2)	
Muscle (Above lateral line)						
A	422.8±19.41 (3)	209.6±19.47 (3)	97.0±10.54 (3)	42.0±4.85 (3)	5.2±0.01 (3)	21.0±2.2
B	480.2±44.68 (3)	184.8±17.70 (2)	86.0±14.8 (2)	44.0±0.50 (2)	7.4±0.40 (2)	
Spleen						
A	4.0±1.44 (3)	2.9±0.27 (3)	0.9±0.23 (3)	0.3±0.04 (3)	0.1±0.01 (3)	0.2±0.05
B	7.9±0.53 (3)	2.0±0.11 (2)	0.8±0.09 (2)	0.4±0.05 (2)	0.1±0.005 (2)	
Carcass						
A***						
B	1,593± (3)	560± (1)	209± (2)	133± (2)	25± (2)	62.0±1.5
Total body burden						
A	2,546.3±73.11	1,042.2±17.98	368.2±11.11	206.9±7.61	36.0±1.15	
B	2,487.7±237.04	922±20.43	347.9±33.49	208.7±8.85	38.1±0.90	

TABLE 4. Mercury concentration (micrograms per gram, wet weight) in the tissues of brook trout: A, exposed for 39 wk to various water concentrations of mercury; B, exposed for 39 wk to various water concentrations of mercury followed by 16 wk in control water. *Mean ±SE; ** number of samples in parentheses.

	Hg water concn (*μg/liter*)					
Tissue	0.93	0.29	0.09	0.03	<0.01 (Control)	Mean concn factor tissue Hg:water Hg
Brain						
A	16.9±1.52* (3)**	5.3±0.20 (3)	1.7±0.11 (3)	0.8±0.04 (3)	0.1±0.04 (3)	2×10^4
B	9.2±0.67 (3)	3.6±0.18 (2)	1.1±0.20 (2)	0.6±0.01 (2)	0.1±0.01 (2)	
Blood cells						
A	46.2±4.62 (3)	21.4±2.91 (3)	5.2±0.27 (3)	2.3±0.36 (3)	0.3±0.01 (3)	6×10^4
B	16.5±2.38 (3)	7.0±0.16 (2)	2.2±0.31 (2)	1.2±0.06 (2)	0.2±0.005 (2)	
Gill						
A	22.2±1.82 (3)	6.2±0.53 (3)	1.6±0.08 (3)	0.7±0.06 (3)	0.1±0.02 (3)	2×10^4
B	7.3±0.47 (3)	1.7±0.13 (2)	0.4±0.09 (2)	0.2±0.03 (2)	0.1±0.00 (2)	
Gonad						
A	12.3±3.73 (3)	2.9±0.53 (3)	0.9±0.41 (3)	0.2±0.01 (3)	0.05±0.00 (3)	1×10^4
B	5.0±0.78 (3)	1.4±0.29 (2)	0.4±0.13 (2)	0.2±0.03 (2)	0.04±0.01 (2)	
Kidney						
A	26.9±5.04 (3)	8.9±1.35 (3)	2.5±0.23 (3)	1.2±0.09 (3)	0.2±0.04 (3)	3×10^4
B	22.9±2.53 (3)	6.0±0.66 (2)	1.8±0.24 (2)	1.1±0.02 (2)	0.4±0.08 (2)	
Liver						
A	24.4±2.37 (3)	8.3±0.34 (3)	2.2±0.15 (3)	0.7±0.06 (3)	0.1±0.02 (3)	2×10^4
B	14.9±1.68 (3)	5.1±0.19 (2)	1.5±0.29 (2)	0.9±0.10 (2)	0.2±0.01 (2)	
Muscle						
A	10.2±1.42 (3)	4.9±0.08 (3)	1.9±0.15 (3)	1.0±0.10 (3)	0.1±0.10 (3)	2×10^4
B	5.6±0.60 (3)	2.3±0.01 (2)	0.8±0.10 (2)	0.4±0.06 (2)	0.1±0.00 (2)	
Spleen						
A	38.7±2.50 (3)	11.8±1.02 (3)	3.2±0.34 (3)	1.2±0.10 (3)	0.2±0.02 (3)	4×10^4
B	24.8±1.96 (3)	6.1±0.16 (2)	1.6±0.16 (2)	0.9±0.03 (2)	0.2±0.005 (2)	
Carcass						
B	4.4± (3)	1.6± (1)	0.59± (2)	0.4± (2)	0.1± (2)	7×10^3
Total body burden						
A	9.4±1.02	3.4±0.17	1.1±0.06	0.7±0.04	0.1±0.01	
B	4.9±0.32	1.8±0.08	0.7±0.09	0.4±0.02	0.1±0 01	2×10^4

lished for muscle alone (Table 4). Whole-body mercury residues in dead fish from unexposed parents were also analyzed. The fish from unexposed parents came from embryos transferred from the control tank at spawning and, since no natural spawning occurred in one of the duplicates at this MMC concentration, exposure of these eggs was not terminated at 4 wk as were all other transferred eggs. Fish from previously exposed parents began to die after 24 wk of continuous exposure and continued to die through the spawning period; all fish from unexposed parents died

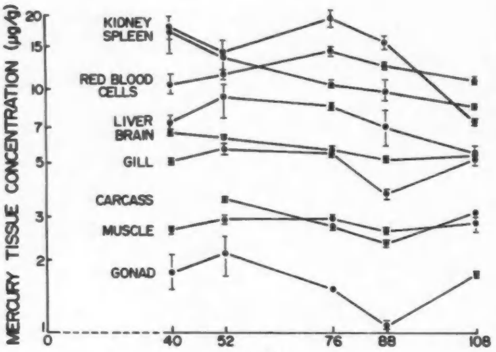

FIG. 5. Distribution of mercury (micrograms Hg per gram wet weight) in the tissues of second-generation brook trout exposed to an MMC water concentration of 0.29 µg Hg/liter. Each point represents the mean and standard deviation of five to six samples.

in 24–28 wk. The concentration of mercury in both living and dead trout from unexposed parents was considerably greater than in trout from exposed parents. The majority of dead second-generation fish from exposed parents contained more mercury (9–10 µg/g) than those surviving (7–8 µg/g). Dead fish from unexposed parents also contained more mercury (15–20 µg/g) than those surviving (11–12 µg/g).

The differences between the survival and residue data on the offspring from exposed and unexposed parents indicate possible acclimation effects.

The distribution of mercury in second-generation brook trout tissues is presented in Fig. 5 for a single MMC water concentration of 0.29 µg Hg/liter, since the tissue distribution of mercury was the same regardless of the water concentrations tested. The general distribution of mercury in these second-generation fish was the same as that described in more detail for first-generation fish (Fig. 2), although the actual concentrations of mercury were less for blood, muscle, and gill, higher for the kidney, and the same for liver, spleen, brain, and gonad. The maximum bioaccumulation found (tissue:water) in second-generation trout during the 108-wk exposure ranged from 6.9×10^3 to 6.3×10^4, depending on the tissue.

Methylmercury vs. total mercury — The amount of mercury present as methylmercury in the muscle tissue of second-generation brook trout after 88 wk of continuous exposure to various water concentrations of MMC was 99.7 ± 6.0% (mean and standard error) when computed by the method of Longbottom et al. (1973) and 90.0 ± 6.0% according to the method of Talmi and Crossman (1973). The percentage of methylmercury in the tissue was always the same regardless of the total amount of mercury present in the tissue.

Discussion

Following the exposure of three generations of brook trout to MMC, no toxic symptoms were apparent at 0.29 µg Hg/liter and below, but definite effects on survival, growth, and reproduction were observed at 0.93 µg Hg/liter and above. Therefore the maximum acceptable toxicant concentration (MATC) of MMC for brook trout is between 0.29 to 0.93 µg Hg/liter, with a calculated application factor (MATC/96-hr LC50) of 0.004 to 0.013. These data agree with those of Matida et al. (1971), who reported deaths and behavioral aberrations in rainbow trout after 10 wk of exposure at 3.7 µg Hg/liter and adverse effects on growth at low concentrations after longer exposures. The most sensitive toxic responses in this study were first indicated during the second-generation 16-wk embryo–alevin–juvenile exposure (Table 2) and were further validated during the remainder of the second- and the third-generation portions of the study. This finding is in agreement with earlier work by McKim and Benoit (1974), in which they showed that an exposure of brook trout yearlings through spawning to 12-wk-old second-generation juveniles was sufficient to predict the effects of low concentrations of copper.

The present study also indicated that brook trout in the early life history stages are more sensitive to MMC than are the older juvenile–adult trout. This early sensitivity has also been shown for other metals and other species of fish (Skidmore 1965, 1966; Hazel and Meith 1970; McKim and Benoit 1971, 1974; Pickering and Gast 1972; Eaton 1974; Pickering 1974).

The rate of mercury uptake from water by brook trout was quite rapid in all tissue studied, and the amount of mercury accumulated was directly proportional to the water MMC concentration. Concentration factors (tissue Hg: water Hg) depended on the tissue in question and ranged from 6.9×10^3 to 6.3×10^4 for both first- and second-generation trout. Second-generation trout exposed to the lowest MMC water concentration for the longest period (36–108 wk) had a muscle (and whole-body) mercury concentration factor of 1.2×10^4. The mercury concentrations in samples of brook trout muscle were essentially the same as whole-body samples, which is understandable, since over half of a fish is muscle tissue. Huckabee et al. (1974) also found this to be true for several fish species sampled from the Great Smoky Mountains. Other researchers have found similar concentration factors for MMC. Reinert et al. (1974) reported a whole-body concentration factor of 8.0×10^3 for rainbow trout after 12 wk of continuous exposure to MMC in a flowing system and described changes in rates of uptake at different temperatures. Hannerz (1968) recorded a maximum concentration factor in northern pike of 1.5×10^3 after 4 wk of exposure, although his exposure consisted of only a single dose of MMC to a natural pond situation.

The steady-state situation seen for residues in both first- and second-generation fish was not caused by a balance between uptake and elimination, since little or no mercury was excreted by the trout (as indicated by the half-life studies in this report), and all fish continued to increase their total mercury content throughout the study. The amount of mercury being continually taken up was balanced against the continual weight gain of the trout. The steady state situation of both first- and second-generation trout seemed to be controlled by this relationship of total micrograms of mercury and total weight.

A considerable amount of mercury was passed on to the embryos from their parents. However, no natural spawning occurred in adults at water concentrations which produced embryo residues that caused deformities, low hatchability, and death. The mercury concentrations measured in alevins at swim-up were as high as any mercury concentrations found throughout the second- and third-generation exposures. These young trout were exposed to mercury transferred to their yolk from their parents and to mercury in the water, both of which contributed to the high concentrations present at this particular stage. At the end of the alevin stage, when they became "free feeding," the rate of mercury accumulation showed a marked downward trend. This was probably caused by the tremendous increase in growth (17 times) between 8 and 20 wk, which exceeded the accumulation rate of the mercury and "diluted" the mercury in the tissues. The upward trend in the mercury accumulation rate between 20 and 28 wk was probably due to a much decreased growth (2.5 times), allowing the accumulation rate of mercury to exceed the growth rate.

The distribution of mercury in the tissues of first-generation brook trout chronically exposed to low concentrations of MMC in this study agreed closely with the tissue distribution of the intravenously injected dose of methylmercuric nitrate given by Backstrom (1969) and with the stomach tube doses of MMC given by Giblin and Massaro (1973) and Miettinen et al. (1970). The route of methylmercury uptake, therefore, seemed to have little effect on the general distribution throughout the fish except for the much lower levels of mercury found in the gills of fish exposed via stomach tube (Miettinen et al. 1970). The distribution of mercury in second-generation brook trout was identical to that in first-generation trout except for an increase in the kidney, which exceeded the amount measured in all the other tissues. Takeda and Ukita (1970) found that the amount of inorganic mercury in the kidney tissue of rats increased with time after the administration of organic mercury. This may explain the high mercury content of the kidney in the second-generation trout, since they were exposed for a much longer period of time allowing for a greater period of demethylation and subsequent accumulation of inorganic mercury in the kidney tissue. The affinity of the kidney for inorganic mercury was further supported by the data of Weisbart (1973) and Backstrom (1969), who exposed several species of fish to inorganic mercury and found that fish kidney had higher concentrations of mercury than any other tissue. We also noted during the mercury-loss studies in first-generation brook trout that the blood was the only tissue analyzed that lost a significant amount of mercury, whereas the kidney was the only tissue to increase its mercury content significantly ($P = 0.05$). This change in mercury content could come about through a metabolic breakdown of the methylmercury in the blood to form inorganic mercury that subsequently moved to the kidney, as was described for mammals by Norseth and Clarkson (1971); or it might simply be due to the redistribution of mercury from the blood to other tissue. A loss from the blood with a redistribution to other tissue after a single intra-gastric dose of MMC to rainbow trout was described by Giblin and Massaro (1973). Burrows and Krenkel (1973) also presented data that indicated possible demethylation of methylmercury in bluegill kidney, although they did not indicate an increase in total mercury in the kidney.

Background levels of mercury in fish will, of course, vary with local conditions, but background concentrations of mercury in the muscle tissue of freshwater fish removed from any known source of mercury contamination have been shown to range between 0.018 and 0.20 μg Hg/g (Lofroth 1969; Kleinert and Degurse 1972;

Sumner et al. 1972; Huckabee et al. 1974). The mercury values determined for muscle samples from control brook trout in this study fell within the range established above for wild fish populations. The mean total mercury concentration of 23.5 μg Hg/g in the muscle (and whole body) tissue of first-generation brook trout that died after 16–28 wk of continuous exposure to the highest MMC water concentration (2.93 μg Hg/liter) was in close agreement with approximately 20 μg Hg/g measured in the tissue of distressed and dying fish taken from the surface of Minamata Bay, Japan, after a spill of MMC from a chemical plant (Kitamura 1968). Second-generation brook trout in the present study also died, but with much lower concentrations of mercury in the tissue (9.5 μg Hg/g) than the first-generation trout, although the exposure time was much longer (64–100 wk) and the water MMC concentration (0.93 μg Hg/liter) was lower. These differences stress the importance of both the tissue concentration and the exposure time in producing toxic symptoms in fish.

The studies described here on the direct toxic effects of MMC on brook trout show that if this form of mercury is present in low concentrations in water, then toxic symptoms ultimately will develop. However, the direct toxicity of MMC was only one aspect of these studies. The bioaccumulation of mercury in the tissues of these trout is perhaps the most important aspect of the study with respect to the environment. In 20–38 wk the lowest water MMC concentration tested (0.03 μg Hg/liter) produced methylmercury residues in the edible portions of all first-generation fish in excess of 0.5 μg Hg/g, the U.S. Food and Drug Administration's "action level" for human consumption (Federal Register 1974). The next higher water concentration (0.09 μg Hg/liter) also produced residues that exceeded 0.5 μg Hg/g in second-generation trout, but the lowest MMC water concentration causing toxic effects on the fish was 0.93 μg Hg/liter. In other words, effects on fish survival, growth, and reproduction were not observed at MMC concentrations that produced methylmercury tissue residues above the U.S. Food and Drug Administration's "action level" for human consumption. No deaths or abnormalities were observed in either first- or second-generation brook trout with whole-body residues of 2.7 μg Hg/g or less, but whole-body residues of 5–7 μg Hg/g eventually caused toxic symptoms and death. Therefore, it might be expected that environmental residues in fish between 3 and 5 μg Hg/g would be possible without direct toxic effects on the fish. Similar results were observed by Olson et al. (1975) who exposed fathead minnows to water MMC concentrations of 0.018–0.247 μg Hg/liter for 48 wk and showed the lowest concentration tested had no effect on survival yet produced whole body residues of 1.47 μg Hg/g. These relatively high tissue residues in fish brought on by exposure to low water concentrations of MMC cause even greater concern when the persistence of mercury in the tissue is considered. The present study showed essentially no loss of mercury from either first- or second-generation brook trout after 12–16 wk in control water. Several other workers have demonstrated both in the field and in the laboratory that fish show a half-life of 1–3 yr for methylmercury (Jarvenpaa et al. 1970; Lockhart et al. 1972; Burrows and Krenkel 1973; W. A. Willford personal communication). These data indicate that elimination of mercury from aquatic animals is extremely slow. Therefore, if aquatic animals are contaminated, many years would be required to completely eliminate the accumulated mercury.

Acknowledgments

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AMERICAN PUBLIC HEALTH ASSOCIATION, AMERICAN WATER WORKS ASSOCIATION, AND WATER POLLUTION CONTROL FEDERATION. 1971. Standard methods for the examination of water and waste water. 13th ed. Washington, D.C. 874 p.

BACKSTROM, J. 1969. Distribution studies of mercuric pesticides in quail and some freshwater fishes. Acta Pharmacol. Toxicol. 27: 1–103.

BENOIT, D. A. 1974. Artificial laboratory spawning substrate for brook trout (*Salvelinus fontinalis* Mitchill). Trans. Am. Fish. Soc. 103: 144–145.

BENOIT, D. A., AND F. A. PUGLISI. 1973. A simplified flow-splitting chamber and siphon for proportional diluters. Water Res. 7: 1915–1916.

BURROWS, W. C., AND P. A. KRENKEL. 1973. Studies on uptake and loss of methylmercury-203 by bluegills (*Lepomis macrochirus* Rafinesque). Environ. Sci. Technol. 7: 1127–1130.

COMMITTEE ON METHODS FOR TOXICITY TESTS WITH AQUATIC ORGANISMS. 1975. Methods for acute toxicity tests with fish, macroinvertebrates, and amphibians. U.S. Environ. Prot. Agency, Ecol. Res. Ser. EPA-660/3-75-009: 67 p.

DRUMMOND, R. A., AND W. F. DAWSON. 1970. An inexpensive method for simulating diel patterns of lighting in the laboratory. Trans. Am. Fish. Soc. 99: 434–435.

EATON, J. G. 1974. Chronic cadmium toxicity to the bluegill (*Lepomis macrochirus* Rafinesque). Trans. Am. Fish. Soc. 103: 729–735.

FEDERAL REGISTER. 1974. Proposed action level for mercury in fish and shell fish. December 6. 122.200. Washington, D.C.

GIBLIN, F. J., AND E. J. MASSARO. 1973. Pharmacodynamics of methylmercury in the rainbow trout (*Salmo gairdneri*): tissue uptake, distribution and excretion. Toxicol. Appl. Pharmacol. 24: 81–91.

HANNERZ, L. 1968. Experimental investigations on the accumulation of mercury in water organisms. Fish. Board Swed. Inst. Freshwater Res. Drottningholm Rep. 48: 120–175.

HAZEL, G. R., AND S. J. MEITH. 1970. Bioassay of king salmon eggs and sac fry in copper solutions. Calif. Fish Game 56: 121–124.

HUCKABEE, J. W., C. FELDMAN, AND Y. TALMI. 1974. Mercury concentrations in fish from the Great Smoky Mountains national park. Anal. Chem. Acta 70: 41–47.

JARVENPAA, T., M. TILLANDER, AND J. K. MIETTINEN. 1970. Methylmercury: half-time of elimination in flounder, pike, and eel. Suom. Kemistil. B 43: 439–442.

KITAMURA, S. 1968. Determination on mercury content in bodies of inhabitants, cats, fishes, and shells in Minamata district and in the mud of Minamata Bay, p. 257–266. In Study group of Minamata disease [ed.] Minamata disease. Kumamato Univ., Japan.

KLEINERT, S. J., AND P. E. DEGURSE. 1972. Mercury levels in Wisconsin fish and wildlife. Wis. Dep. Nat. Res., Wis. Tech. Bull. 52 (Res. Rep. 74): 22 p.

KOPP, J. F., M. C. LONGBOTTOM, AND L. B. LOBRING. 1972. "Cold vapor" method for determining mercury. J. Am. Water Works Assoc. 64: 20.

LOCKHART, W. L., J. F. UTHE, A. R. KENNEY, AND P. M. MEHRLE. 1972. Methylmercury in northern pike (*Esox lucius*): distribution, elimination, and some biochemical characteristics of contaminated fish. J. Fish. Res. Board Can. 29: 1519–1523.

LOFROTH, G. 1969. Methylmercury. Rep. Radiobiol. Div., Dep. Biochem., Univ. Stockholm, Work. Group Environ. Toxicol. Ecol. Res. Comm., Swed. Natl. Sci. Res. Counc., Stockholm, Swed. 38 p.

LONGBOTTOM, J. J., R. C. DRESSMAN, AND J. J. LICHTENBERG. 1973. Gas chromatographic determination of methylmercury in fish, sediment, and water. J. Ass. Off. Anal. Chem. 56: 1297–1303.

MATIDA, Y., H. KUMADA, S. KIMURA, Y. SAIGA, T. NOSE, M. YOKOTE, AND H. KAWATSU. 1971. Toxicity of mercury compounds to aquatic organisms and accumulation of the compounds by the organisms. Bull. Freshwater Fish. Res. Lab. (Jpn.) 21: 197–227.

MCALLISTER, W. A., W. L. MAUCK, AND F. L. MAYER. 1972. A simplified device for metering chemicals in intermittent-flow bioassays. Trans. Am. Fish. Soc. 101: 555.

MCKIM, J. M., AND D. A. BENOIT. 1971. Effects of long-term exposures to copper on survival, growth, and reproduction of brook trout (*Salvelinus fontinalis*). J. Fish. Res. Board Can. 28: 655–662.

——— 1974. Duration of toxicity tests for establishing "no effect" concentrations for copper with brook trout (*Salvelinus fontinalis*). J. Fish. Res. Board Can. 31: 449–452.

MIETTINEN, V., E. BLANKENSTEIN, K. RISSANEN, M. TILLANDER, AND J. K. MIETTINEN. 1970. Preliminary study on the distribution and effects of two chemical forms of methyl mercury in pike and rainbow trout. FAO technical conference on marine pollution and its effects on living resources and fishing, Rome, Italy. p. 1–12.

MOUNT, D. I., AND W. A. BRUNGS. 1967. A simplified dosing apparatus for fish toxicology studies. Water Res. 1: 21–29.

MOUNT, D. I., AND C. E. STEPHAN. 1967. A method for establishing acceptable toxicant limits for fish-malathion and the butoxethanol ester of 2,4-D. Trans. Am. Fish. Soc. 96: 185–193.

NORSETH, T., AND T. S. CLARKSON. 1971. Intestinal transport of 203 Hg-labelled methylmercury chloride. Arch. Environ. Health 22: 568–577.

OLSON, G. F., D. I. MOUNT, V. M. SNARSKI, AND T. W. THORSLUND. 1975. Mercury residues in fathead minnows (*Pimephales promelas* Raf.), chronically exposed to methylmercury in water. Bull. Environ. Contam. Toxicol. 14: 129–134.

PICKERING, Q. H. 1974. Chronic toxicity of nickel to the fathead minnow. J. Water Pollut. Control Fed. 46: 760–765.

PICKERING, Q. H., AND M. H. GAST. 1972. Acute and chronic toxicity of cadmium to the fathead minnow (*Pimephales promelas*). J. Fish. Res. Board Can. 29: 1099–1106.

REINERT, R. E., L. J. STONE, AND W. A. WILLFORD. 1974. Effect of temperature on accumulation of methylmercuric chloride and p,p'DDT by rainbow trout (*Salmo gairdneri*). J. Fish. Res. Board Can. 31: 1649.

SKIDMORE, J. F. 1965. Resistance to zinc sulphate of the zebra fish (*Brachydania rerio*) at different phases of its life history. Ann. Appl. Biol. 56: 47–53.

——— 1966. Resistance to zinc sulphate of zebra fish (*Brachydanio rerio*) embryos after removal or rupture of the outer egg membrane. J. Fish. Res. Board Can. 23: 1037–1042.

SPRAGUE, J. G. 1969. Measurement of pollutant toxicity to fish. 1. Bioassay methods for acute toxicity. Water Res. 3: 793–821.

STEEL, R. G. D., AND J. H. TORRIE. 1960. Principles and procedures of statistics. McGraw-Hill, Inc., New York, N.Y. 481 p.

SUMNER, A. K., J. G. SAHA, AND Y. W. LEE. 1972. Mercury residues in fish from Saskatchewan waters with and without known sources of pollution-1970. Pestic. Monit. J. 6: 122–125.

TAKEDA, Y., AND T. UKITA. 1970. Metabolism of ethylmercuric chloride-203 Hg in rats. Toxicol. Appl. Pharmacol. 17: 181–188.

TALMI, Y., AND R. CROSSMAN. 1973. Separation, detection, and identification of organically bound toxic metals, and other hazardous materials. Ecol. Anal. Trace Contam. Oak Ridge National Laboratory-NSF-EATC-1: p. 287–307.

WEISBART, M. 1973. The distribution and tissue retention of mercury-203 in the goldfish (*Carassius auratus*). Can. J. Zool. 51: 143–150.

Evaluation of a Toxicant on the Metabolism of Model Stream Communities[1,2]

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MAKI, A. W., AND H. E. JOHNSON. 1976. Evaluation of a toxicant on the metabolism of model stream communities. J. Fish. Res. Board Can. 33: 2740–2746.

The effects of a toxicant, the lampricide TFM (3-trifluoromethyl-4-nitrophenol), on the metabolism of benthic communities were studied in a series of six indoor model streams resembling typical woodland streams. Each artificially illuminated stream consisted of a 4-m pool section and a 4-m riffle section.

A specially developed in situ stream respirometer was used for measurements of net primary production and community respiration in pool and riffle communities. Pretreatment levels of gross primary production ranged during summer, fall, and early winter from 10.7 to 79.0 mg $O_2 \cdot m^{-2} \cdot h^{-1}$ and were suppressed by 25–50% during exposure to 9.0 mg/liter TFM. Community respiration ranged from 10.5 to 36.2 mg $O_2 \cdot m^{-2} \cdot h^{-1}$ during the same time period and was increased 3–50% by the 9.0 mg/liter lampricide treatment. Calculated photosynthesis to respiration (P:R) ratios proved to be sensitive indicators of the influence of the toxicant. The stream communities demonstrated a capacity to adjust to the toxicant influence as evidenced by the rapid return of metabolic rates to pretreatment levels following the exposure period.

MAKI, A. W., AND H. E. JOHNSON. 1976. Evaluation of a toxicant on the metabolism of model stream communities. J. Fish. Res. Board Can. 33: 2740–2746.

Les auteurs ont étudié les effets d'un toxique, le lampricide TFM (3-trifluorométhyl-4-nitrophénol), sur le métabolisme de communautés benthiques dans une série de six cours d'eau modèles intérieurs ressemblent à des cours d'eau sous bois typiques. Chaque cours d'eau éclairé artificiellement comprenait une section de 4 m en fosse et une section de 4 m en radier.

Un respiratomètre in situ spécialement conçu a été utilisé pour mesurer la production primaire nette et la respiration des communautés des fosses et des radiers. Les niveaux d'avant-traitement de production primaire brute varient pendant les mois d'été, d'automne et du début de l'hiver de 10.7 à 79.0 mg $O_2 \cdot m^{-2} \cdot h^{-1}$ et baissent de 25–50% pendant une exposition à 9.0 mg/litre de TFM. La respiration des communautés varie de 10.5 à 36.2 mg $O_2 \cdot m^{-2} \cdot h^{-1}$ durant la même période et augmentent de 3–50% lors du traitement à 9.0 mg/litre de lampricide. Les quotients calculés (P:R) s'avèrent des indicateurs sensibles de l'influence du toxique, et les communautés fluviatiles démontrent une aptitude à s'ajuster à l'influence eu toxique, comme l'indique le rapide retour des taux métaboliques aux niveaux d'avant-traitement aprés la période d'exposition.

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EFFECTS of a particular environmental contaminant are generally assessed on a single organism existing in isolated aquaria or toxicity test chambers for set periods of time. A species exists in nature as an integral part of the ecosystem, exerting effects and being affected by the interactions of the natural community. Rarely have investigators taken into account the influence of the interactions of community structure when assessing the effects of a toxicant or pollutant on an aquatic species (Williams 1969; Seim 1970; Cairns et al. 1972; Aubert et al. 1972).

Measurements of changes in the metabolic rate of stream and river communities have generally been used to characterize changes in the stream community brought about by nutrient inputs and cultural eutrophication (Odum 1956; Cole 1973). These changes are generally of a long-term nature and their measurement involves comparisons with similar upstream stations for control data. As models of small natural streams, artificial streams employing constraints on the complexity of communities have been used by several investigators to observe interactions between individuals and populations and thus to describe the operations

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of the community and existing trophic levels (Kevern and Ball 1965; McIntyre and Phinney 1965; Davis and Warren 1968).

This community approach was used in the current investigation to examine the toxic influence of larval lampricide (TFM, 3-trifluormethyl-4-nitrophenol) on the production and metabolism of replicated model stream communities. An extensive review of the literature concerning safety and application of TFM in surface waters was published by Schnick (1972). By imposing constraints on the physical and biological complexity of the model stream population, the description of the community interactions under the influence of the toxicant is simplified (Warren and Davis 1971). This paper is an attempt to add an additional dimension to currently existing techniques for quantitative analysis of community structure and environmental assessments by evaluating toxic effects on the total functional ability of the integral community.

Materials and Methods

MODEL STREAMS

The site selected for the construction of the model streams was the Department of Natural Resources fish hatchery located in Mecosta County at Paris, Michigan. Six hatchery channels, 8 m long and 0.6 m wide, were utilized for the model stream communities, providing for three complete systems each consisting of one control channel with pool and riffle and an adjacent experimental channel, also with pool and riffle areas. The upstream channels were designated pool communities and maintained at a 25.4-cm water depth. Colonization of these pool areas began in the fall of 1972 through input of organic matter and drifting insect larvae entering through the gravity-fed water intake on adjacent Cheney Creek.

In early May natural stream substrate with associated flora and fauna was removed from Buckhorn Creek, a similar spring-fed stream 0.4 km north of the hatchery, and trucked to the model streams. Gravel and small rubble up to 10-cm diam were added to each riffle section providing depth of 15–20 cm of substrate and approximately 8 cm of water. Care was taken to insure a uniform distribution of particle sizes between streams. An 8-wk period was allotted for stabilization and growth of these riffle communities before experimentation was initiated in late June. A complete listing of the flora and fauna that developed within the model system is presented in Table 1.

Water supplied to each channel passed through the length of the model stream only once and was collected into common floor drains and discharged through a 25-cm pipe back into Cheney Creek downstream from the hatchery building. The standing volume of a single stream, including riffle and pool sections, was approximately 550 liters. At a flow rate of 100 liters/min per channel, the time for total water replacement volume was about 5.5 min. The water quality remained relatively constant throughout the year with an average alkalinity of 179 mg/liter $CaCO_3$, pH of 7.8, and average hardness of 211 mg/liter. Water temperature reflected the spring origin of the water, never exceeding 13 C during summer and dropping to 5 C in early December. Lighting, supplied to the model streams in late April 1973, was in the form of 24 banks of four high intensity cool-white fluorescent lamps. The lamps were attached to sheets of 1-cm plywood painted white and provided with chains for raising or lowering the lamps to adjust the surface intensity of light. All lamps were placed to provide an intensity of 9688 ± 538 lx at the water surface and a photoperiod to conform to natural day length and seasonal adjustments.

PHOTOSYNTHESIS–RESPIRATION CHAMBER

Rates of photosynthesis and respiration by intact benthic communities were monitored by the use of a modified photosynthesis–respiration chamber as described by D. King (Kellogg Biological Station, Michigan State University, personal communication). Thirty-six chambers consisting of a circular plexiglass tray of 28-cm internal diam were placed in the hatchery channels prior to addition of substrate and colonization. A tight-fitting circular top section of 29-cm internal diam which fit down around the buried bottom section effectively sealed the enclosed community (Fig. 1). The lid was provided with a centrally located 4-cm port to accommodate a Yellow Springs Instruments Model 5420A Self-Stirring B.O.D. Oxygen/Temperature Probe. The probe was connected to a YSI Model 54 Oxygen Meter designed to read directly in parts per million and compensated for temperature effects on membrane permeability and oxygen solubility in water. A continuous record of oxygen concentration was provided by Sargent-Welch Model SR Strip Chart Recorders.

EXPERIMENTAL PROCEDURE

Prior to an experiment, a chamber top was fit down over the outside of the bottom counterpart avoiding entrainment of air bubbles and insuring a watertight seal. The height, and consequently the volume, of the chamber was adjusted to equal the water depth of the stream outside the chamber to insure that the temperature inside the chamber would remain equal to the ambient stream, thereby avoiding temperature-induced changes on the metabolic rates of the enclosed organisms during the experiment. The chamber volumes ranged from 3500 ml in the riffles to 4500 ml in the pools. The stirring rod of the BOD (biochemical oxygen demand) probe provided a continuous circular current within the confines of the photosynthesis–respiration chamber to stimulate stream flow.

An experiment with a single chamber typically lasted 5½–6 days to allow for 2 days of pretreatment data, 1 complete day under exposure to 9.0 mg/liter

TABLE 1. Taxonomic listing of flora and fauna of indoor model streams at Paris, Michigan 1973–74.

Macroinvertebrates	Filamentous Green Algae	Diatoms
Amphipoda	*Stigeoclonium tenue*	*Tabellaria* sp.
Gammarus pseudolimnaeus	*Spirogyra* sp.	*Cocconeis* sp.
Isopoda	*Cladophora* sp.	*Diatoma* sp.
Asellus militaris	*Rhizoclonium* sp.	*Achnanthes* sp.
		Nitzschia sp.
Plecoptera		*Meridion* sp.
Paracapnia sp.		*Fragilaria* sp.
Amphinemura varshava		*Synedra* sp.
Leuctra tenuis		*Navicula* sp.
Phasganophora sp.		*Eunotia* sp.
Acroneuria lycorias		*Pinnularia* sp.
Ephemeroptera		*Gyrosigma* sp.
Ephemerella lata		
Paraleptophlebia mollis		
Baetis sp.		
Trichoptera		
Hydropsyche sp.		
Cheumatopsyche sp.		
Goera calcarata		
Glossosoma sp.		
Neophylax sp.		
Limnephilus sp.		
Brachycentrus americanus		
Chimarra obscura		
Ochrotrichia sp.		
Diptera		
Simulium sp.		
Antocha sp.		
Chrysops sp.		
Prodiamesa sp.		
Diamesa sp.		
Cricotopus sp.		
Eukiefferiella sp.		
Tanytarsus sp.		
Chironomus sp.		
Miscellaneous		
Sialis sp.		
Stenelmis sp.		
Hydracarina sp.		
Physa sp.		
Lymnaea sp.		
Annelida		

active TFM in Field Grade formulation injected via a syringe through the rubber stopper, and 2 days of postexposure data. The 9 mg/liter concentration was precalculated from the results of static toxicity tests with lamprey as the level that would be used during actual lamprey control treatments in Cheney Creek water. Each chamber was opened, allowed to rinse with stream water, and refilled at least every 24 h to eliminate metabolic effects due to concentration of metabolites within the enclosed community and to renew nutrient concentrations. Several experiments particularly in the strongly heterotrophic pool communities required rinsing and refilling at intervals of 5–6 h since oxygen concentrations would drop below 5 mg/liter. Also, during the summer when there was a peak standing biomass of *Cladophora* and *Stigeoclonium tenue* in the riffle communities, supersaturation with oxygen and the formation of oxygen bubbles in the chamber during photosynthesis required more frequent rinsing and refilling of the chamber.

Measurements of net photosynthesis were made during the daylight hours under constant 9688 lx illumination and measurements of community res-

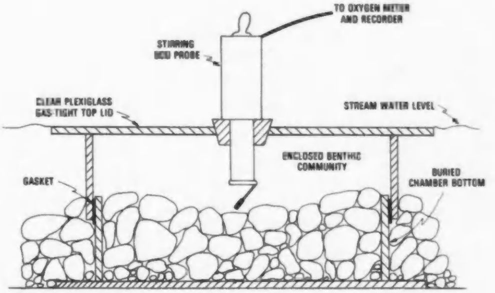
TO OXYGEN METER AND RECORDER
STIRRING BOD PROBE
CLEAR PLEXIGLASS GAS-TIGHT TOP LID
STREAM WATER LEVEL
ENCLOSED BENTHIC COMMUNITY
GASKET
BURIED CHAMBER BOTTOM
CONCRETE CHANNEL BOTTOM

FIG. 1. Cross-sectional diagram of photosynthesis–respiration chamber in operation on stream bottom.

piration were made on the same community during the night. All rates were then calculated from the calibrated strip-chart recorder paper and plant and animal respiration were subtracted to yield net primary production.

Results

The estimates of community metabolism were calculated directly from the diurnal changes in oxygen concentration after Whitworth and Lane (1969). Negative values for oxygen production frequently were obtained in the strongly heterotrophic pool communities. For example, an oxygen concentration of 9.8 mg/liter at sunrise may drop to 6.4 mg/liter during the course of the day under full illumination resulting in a negative value for net community production. The rate of loss was calculated and compared with the rate of oxygen depletion for that same community during the night. If this nighttime rate was greater than the daytime rate of loss, as was generally the case during the summer, the difference between these day and night rates was interpreted to be due to the oxygen production by diatoms and filamentous green algae on the surface of the pool community; this was recorded as daytime gross production. However, during the exposure to 9.0 mg/liter TFM, there was generally no detectable difference between rates of oxygen depletion in the pools during day and night. In these instances the gross production was recorded as zero, the rate of oxygen depletion as respiration, and the P:R ratio as zero.

The summary calculations of primary production and community respiration for a typical 5-day experiment with two particular chambers located in riffle and pool communities of one stream are presented graphically in Fig. 2 and 3. These rates for late August are among the highest values for gross primary production achieved in the riffle communities during the year. The high production of about 75 mg $O_2 \cdot m^{-2} \cdot h^{-1}$ are due to the large standing crop of filamentous green algae, primarily *Cladophora* sp. and *Stigeoclonium* sp., present at that time of year.

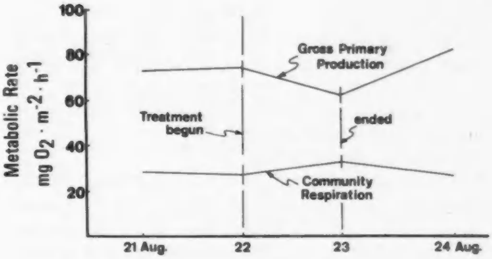

FIG. 2. Community metabolism in stream experimental riffle during August 21–24, 1973.

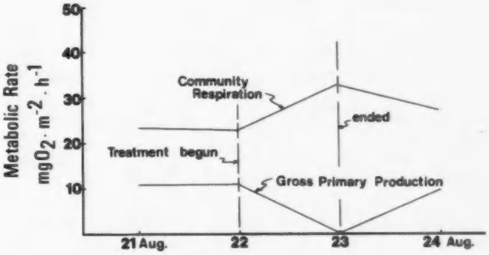

FIG. 3. Community metabolism in stream experimental pool during August 21–24, 1973.

The characteristic influence of the 9.0 mg/liter TFM exposure is represented by the respirometry data for August 23. Under these conditions the rate of gross primary production dropped to a low of 62 mg $O_2 \cdot m^{-2} \cdot h^{-1}$ in the riffle and to undetectable levels in the pool, while the respiration rates of the enclosed riffle and pool communities increased to 29 and 34 mg $O_2 \cdot m^{-2} \cdot h^{-1}$, respectively. The depressed evolution of oxygen by the algal community reflects the algistatic effect of TFM as demonstrated in laboratory toxicity tests (Maki et al. 1975) and the increased respiration rates are believed to be primarily due to the response of the macroinvertebrates to the presence of TFM. The increased respiration rate of invertebrates is recognized as a metabolic response to stress agents (Whitley and Sikora 1970).

Values for gross primary production in the riffles ranged from approximately 58.2 to 79.0 mg $O_2 \cdot m^{-2} \cdot h^{-1}$ during the summer to winter period (Fig. 4). Highest rates were recorded during the late summer and late fall periods which presumably were brought about by the increase in biomass of filamentous greens and attached diatoms, respectively. The lowest production rates were recorded between these periods during the time when the communities were in a state of change, i.e. newly developed growths of green algae or sloughing of senescent growths of green algae accompanied by increases in the abundance of diatoms.

For each experimental exposure throughout the year, the rate of oxygen evolution was decreased by the addition of TFM (Fig. 4). The amount of this departure presumably reflects the species composition of the primary producers. The gross production rate was depressed by about 5–10% during summer when the producer community was dominated by filamentous greens; however, in the later fall months when the producer community was composed primarily of attached diatoms, the depression of oxygen production averaged 25–50%. In laboratory toxicity tests diatoms were found to be more susceptible to TFM (96 h EC50: 1.5–3.0 mg/liter) than the green algae (96 h EC50: 5.0–7.9 mg/liter), (Maki et al. 1975). The rates of gross primary production of the fall diatom communities were obviously much more influenced by the TFM exposure than were the production rates of the summer filamentous green communities.

The strongly heterotrophic nature of the pool communities throughout the year is shown in Fig. 5. Average values for gross primary production ranged from 10.7 to 22.5 mg $O_2 \cdot m^{-2} \cdot h^{-1}$ during the summer and were primarily due to a low density of filamentous greens covering the surface of the organic detritus in the pools. As in the riffle communities, these populations disappeared from the model streams in early fall and positive values for oxygen production in the pools were not detected beyond this date. The response of these communities to TFM was also similar, showing a depression of oxygen evolution in all experiments.

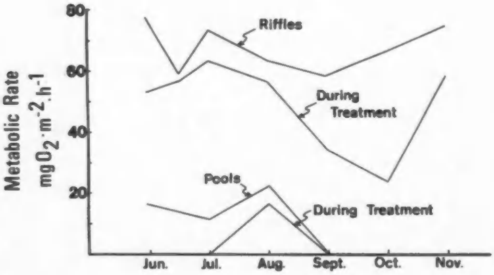

FIG. 4. Rates of gross primary production recorded throughout 1973 under a daytime illumination of 9688 lx.

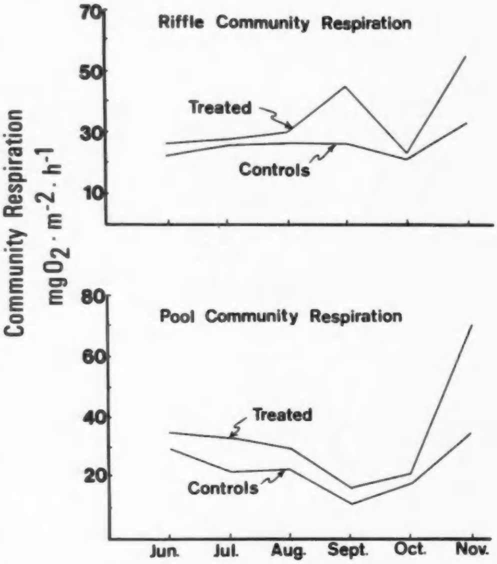

FIG. 5. Rates of community respiration observed in riffle and pool areas throughout 1973.

The temporary nature of the effects of TFM was demonstrated by the posttreatment experiments. Values for gross primary production in the riffle and pool communities returned to pretreatment rates within 24–36 h postexposure. Statistical analysis of the pretreatment riffle community rates of gross primary production and the during treatment rates demonstrated a significant difference at the 0.01 level, whereas the pool communities demonstrated a difference at the 0.02 level between pretreatment and during treatment rates. No significant difference was found between pre- and posttreatment rates for either community (Table 2).

The pretreatment respiration values for the riffle communities ranged from 20.3 to 32.4 mg $O_2 \cdot m^{-2} \cdot h^{-1}$ and increased to 22.0–60.7 mg $O_2 \cdot m^{-2} \cdot h^{-1}$ under the exposure to 9.0 mg/liter TFM (Fig. 5). This represents an increase of 3–50% due to the toxicant. Similar results were obtained in the pool communities where the high density of burrowing midge larvae was primarily responsible for the increased respiration values obtained in the presence of TFM. Normal values for respiration in the pools ranged from 10.5 to 36.2 mg $O_2 \cdot m^{-2} \cdot h^{-1}$ and were increased to 15.5–73.5 mg $O_2 \cdot m^{-2} \cdot h^{-1}$ by the exposure to TFM. Respiration results were generally more variable than production measurements between the sampling dates presumably due to emergence

TABLE 2. Mean values of community metabolism data during the control period, on exposure to larvicide, and after larvicide treatment. Levels of significant differences determined by Students' *t*-test are given in parentheses.

Community	Pretreatment control	Exposed to 9.0 mg/liter TFM	Posttreatment
	Gross primary production (*mg* $O_2 \cdot m^{-2} \cdot h^{-1}$)		
Riffle	66.08	46.83 (0.01)	61.40 (1.0)
Pool	26.48	11.84 (0.02)	21.64 (1.0)
	Respiration (*mg* $O_2 \cdot m^{-2} \cdot h^{-1}$)		
Riffle	26.03	37.74 (0.2)	23.41 (1.0)
Pool	22.36	30.49 (0.2)	20.00 (1.0)
	P:R ratio		
Riffle	1.36	0.79 (0.01)	1.38 (1.0)
Pool	0.39	0.15 (0.01)	0.29 (0.2)

of species during the year. Posttreatment experiments indicated a rapid recovery and return to pretreatment respiration rates within 24–36 h. Differences in mean riffle and pool respiration rates measured before and during treatment were significant at only the 0.2 level and no statistical difference was found between pre- and posttreatment rates (Table 2).

The most sensitive indicator of TFM effects on community metabolism was the difference between P:R ratios obtained during pre- and posttreatment tests and those obtained during exposure to the 9.0 mg/liter TFM in the riffles (Fig. 6). Since the action of TFM is to decrease oxygen production values and increase respiration values, the P:R ratio is subject to large changes in the presence of the toxicant. The mean ratios of pre- and posttreatment experiments ranged from 1.07 to 1.78, while those obtained under exposure to TFM varied from 0.5 to 1.27. In most experiments, the P:R ratio for the pool communities approached zero and averaged an approximate 50% decrease in the remaining experiments. Analysis of the differences between pre- and posttreatment means and the during-treatment means for the riffle and pool communities indicated differences significant at the 0.01 level (Table 2).

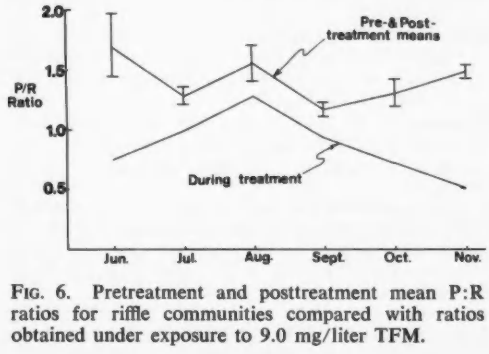

FIG. 6. Pretreatment and posttreatment mean P:R ratios for riffle communities compared with ratios obtained under exposure to 9.0 mg/liter TFM.

Discussion

By measuring community metabolism we determined that respirometry measurements could provide a short-term assessment of the influence of TFM lampricide on the stream community. Since the compound has been shown to be strongly algi-static at relatively low exposure levels (Maki et al. 1975), we hypothesized that sublethal effects on algal metabolism may be produced during a short-term exposure. This hypothesis was also supported by the work of Huang and Gloyna (1968) who tested the effects of numerous phenolic compounds on photosynthetic oxygen production. Using a direct manometric technique, they found that halogenophenols exhibited greater destructive effects than the unsubstituted phenol and that introduction of a nitro group to phenol greatly decreased the chlorophyll content of exposed cultures. They found that the *o*-, *m*- and *p*-nitrophenols reduced the rate of photosynthetic oxygen production by as much as 70% at relatively high exposure levels of 50–100 mg/liter.

Although our values for gross primary production in the riffle communities were considerably lower than those reported by McIntyre and Phinney (1965), the greater illumination intensities, warmer temperatures, and extremely high standing-crop figures for the primary producers in our streams make comparisons invalid. Reese (1966) obtained measurements of primary production in nonenriched sections of a shallow woodland stream ranging from 52 to 200 mg $O_2 \cdot m^{-2} \cdot h^{-1}$. Since the intent of this study was to model a small woodland stream, favorable comparisons can be made. Under an exposure to 9.0 mg/liter TFM the gross primary production dropped from 5 to 50% of the pretreatment rates in the riffle communities and was reduced to nondetectable levels in the pool areas.

Pretreatment rates of community respiration ranged from 20.3 to 33.1 mg $O_2 \cdot m^{-2} \cdot h^{-1}$ in the riffle areas and from 10.2 to 54.0 mg $O_2 \cdot m^{-2} \cdot h^{-1}$ in the pool areas. These values are again lower than the range of 104–171 mg $O_2 \cdot m^{-2} \cdot h^{-1}$ reported by McIntire and Phinney, also probably due to the lower standing crop of primary producers in this study. The values compare favorably with the range of 16–125 mg $O_2 \cdot m^{-2} \cdot h^{-1}$ determined by Williams (1969) for laboratory streams and Reese (1966) for small woodland streams. Exposure of the communities in this investigation to 9.0 mg/liter TFM consistently caused an increase in respiration rates from 5 to 40% in the riffle areas and from 10 to 50% in the heterotrophic pool areas. This is undoubtedly a measure of the increased respiration rates of the enclosed macroinvertebrate community as a response to the sublethal stress of the toxicant exposure.

The P:R ratio is an important tool and a sensitive indicator of subacute stress on the functioning of an intact stream community. The P:R ratios calculated in this study ranged from 0.92 to 2.07 in the riffle communities and from 0.0 to 0.68 in the pool communities. The P:R ratio is an excellent measure of the degree of heterotrophy of a community; generally, the lower the ratios, the more strongly the heterotrophic conditions as shown by the calculated ratios for the pool areas. The data again compare favorably with Reese (1966) where P:R ratios ranged from 0.16 to 0.85 and on average were slightly lower than the P:R ratio of 2.3 calculated by McIntire and Phinney (1965). The P:R ratio was found to be the most sensitive indicator of the toxic influence of TFM where values calculated for communities under exposure to the toxicant dropped from 0.5 to 1.0 compared with pretreatment ratios. In most instances the effects of the lampricide were sufficient to drive gross production in the pool communities to undetectable levels which then produced a P:R ratio of zero.

The capacity of the stream ecosystem to adjust to the temporary toxic effects of TFM is demonstrated in the posttreatment data and should be emphasized. In pool and riffle areas examined, the gross production, respiration, and P:R ratios measured within 1 or 2 days following the day of exposure had returned to pretreatment levels with no significant differences detectable.

Acknowledgments

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AUBERT, M., R. BITTEL, F. LAUMOND, M. ROMEO, B. DONNIER, AND M. BARELLI. 1972. Utilisation d'une chaine trophodynamique de type pelagigue pour l'etude des transferts des pollutions metalliques. Rev. Int. Oceanogr. Med. 28: 27–52.

CAIRNS, J. R., R. LANZA, AND B. C. PARKER. 1972. Pollution related structural and functional changes in aquatic communities with emphasis on freshwater algae and protozoa. Proc. Acad. Nat. Sci. Philadelphia 124: 79–127.

COLE, R. A. 1973. Stream community response to nutrient enrichment. J. Water Pollut. Control Fed. 45: 1874–1888.

DAVIS, G. E., AND C. E. WARREN. 1968. Estimation of food consumption rates, p. 227–248. *In* W. E. Ricker [ed.] Methods for assessment of fish production in fresh waters. IBP Handb. 3. Blackwell Scientific Publications, Oxford. 313 p.

HUANG, J.-C., AND E. F. GLOYNA. 1968. Effect of organic compounds on photosynthetic oxygenation I. Chlorophyll destruction and suppression of photosynthetic oxygen production. Water Res. 2: 347–366.

KEVERN, N. R., AND R. C. BALL. 1965. Primary productivity and energy relationships in artificial streams. Limnol. Oceanogr. 10: 74–87.

MAKI, A. W., L. W. GEISSEL, AND H. E. JOHNSON. 1975. Toxicity of TFM (lampricide) to 10 species of algae. U.S. Bur. Sport Fish. Wildl. Invest. Fish Control 56: 17 p.

MCINTIRE, C. D., AND H. K. PHINNEY. 1965. Laboratory studies of periphyton production and community metabolism in lotic environments. Ecol. Monogr. 35: 237–258.

ODUM, H. T. 1956. Primary production in flowing waters. Limnol. Oceanogr. 1: 102–117.

REESE, H. W. 1966. Physiological ecology and structure of benthic communities in a woodland stream. Ph.D. Thesis. Oregon State Univ., Corvallis, Oreg. 134 p.

SCHNICK, R. A. 1972. A review of the literature on TFM (3-trifluormethyl-4-nitrophenol) as a lamprey larvicide. U.S. Bur. Sport Fish. Wildl. Invest. Fish Control 44: 31 p.

SEIM, W. K. 1970. Influence of biologically stabilized kraft mill effluent on the food relations and production of juvenile chinook salmon in laboratory streams. M.Sc. Thesis. Oregon State Univ., Corvallis, Oreg. 55 p.

WARREN, C. E., AND G. E. DAVIS. 1971. Laboratory stream research: objectives, possibilities and constraints. Annu. Rev. Ecol. Syst. 2: 111–144.

WHITLEY, L. S., AND R. A. SIKORA. 1970. The effect of three common pollutants on the respiration rate of tubificid worms. J. Water Pollut. Control Fed. 42: 57–66.

WHITWORTH, W. R., AND T. H. LANE. 1969. Effects of toxicants on community metabolism in pools. Limnol. Oceanogr. 14: 53–58.

WILLIAMS, H. 1969. Effect of kraft mill effluent on structure and function of periphyton communities. Ph.D. Thesis. Oregon State Univ., Corvallis, Oreg. 117 p.

Nutrients, Chlorophyll, and Internal Tides in the St. Lawrence Estuary[1]

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THERRIAULT, J.-C., AND G. LACROIX. 1976. Nutrients, chlorophyll, and internal tides in the St. Lawrence estuary. J. Fish. Res. Board Can. 33: 2747–2757.

Tide-dependent variations of temperature, salinity, dissolved oxygen, phosphate, nitrate, and chlorophyll *a* support the existence of internal tides (longitudinal and transversal) in the St. Lawrence estuary. Vertical oscillations of the poorly oxygenated and nutrient-rich intermediate and deep waters of the estuary have been documented at the head of the Laurentian Channel, the region in which the internal tides are thought to be generated. Penetration of intermediate waters (high-nutrient and low-oxygen concentrations) beyond the Laurentian Channel associated with the internal tides and linked with an intense mixing process in the upstream region permits the nutrient enrichment of the surface waters and their eventual advection in the seaward direction.

THERRIAULT, J.-C., AND G. LACROIX. 1976. Nutrients, chlorophyll, and internal tides in the St. Lawrence estuary. J. Fish. Res. Board Can. 33: 2747–2757.

Des observations sur les variations de la température, de la teneur en oxygène dissous, des concentrations de sels nutritifs et de la chlorophylle *a*, en fonction de la marée, ont permis d'apporter de nouveaux faits appuyant l'existence et identifiant le lieu de génération de marées internes longitudinales et transversales dans l'estuaire du Saint-Laurent. Celles-ci prennent naissance à la tête du Chenal laurentien et impliquent ses eaux profondes et intermediaires, faiblement oxygénées et riches en sels nutritifs. À ces marées internes, est liée la pénétration, en aval, des eaux intermédiaires (riches en sels nutritifs) du Chenal laurentien. Un mécanisme de mélange vertical d'intensité variable permet ensuite l'enrichissement des eaux de surface en phosphates et en nitrates et leur éventuel transport vers l'aval de l'estuaire.

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THE most striking bathymetric feature of the St. Lawrence estuary is the Laurentian Channel, which exceeds 300 m in depth. It extends from the Atlantic Ocean into the estuary to the confluence of the Saguenay and St. Lawrence rivers, where the bottom rises very steeply from a depth of approximately 350 m to 25 m over a 16-km distance (Fig. 1). Consequently, the circulation of the water masses in the region is very complex.

Huntsman (1923) was the first to suspect the existence of anomalies in this region. He suggested that the combined effect of the inward–outward flow of the tide produces a net flow of the surface waters in the seaward direction, with a compensating movement of the deep water toward the head of the Laurentian Channel and an upwelling in the surface water near Ilet Rouge. During the flood tide, the inward movement of the deep water and the upwelling at the surface are strong, and the outward movement of the surface water is weak or even reversed. During the ebb tide, the outward advection of the surface water is intense, whereas the other two movements are relatively weaker. The comprehensive survey of surface currents published by the Canadian Hydrographic Service (1939) supported Huntsman's hypothesis. Dugal (1934), Gaudry (1938), Nadeau (1938), and Vachon et al. (1938) also observed several anomalies in the surface waters and tried to explain them by hypothesizing an upwelling of deep water. Recently, Steven (1974) described this region of the estuary as a "nutrient pump" but without suggesting any causative mechanism.

Observations of currents, temperature, and salinity made by W. D. Forrester (unpublished data) on a transect near Pointe-au-Père have shown the presence of an internal oscillation in the estuary. He found that this oscillation is composed of a cross-channel standing wave of

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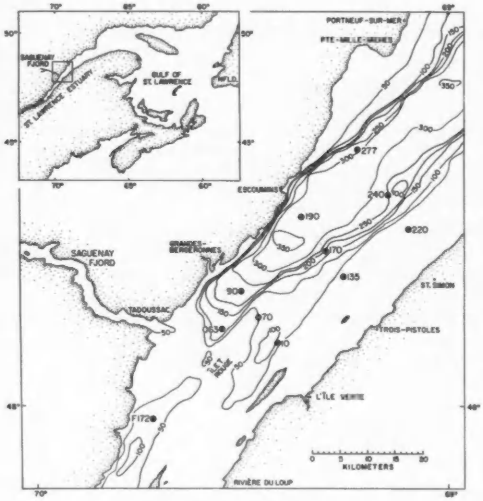

FIG. 1. Location of the stations in the St. Lawrence estuary. The isobaths are in meters.

semidiurnal period with a wavelength twice the channel width, associated with a progressive longitudinal wave that could not be characterized completely by his data. Further measurements of currents between Trois-Pistoles and Baie-Comeau (Forrester 1974) confirmed the presence of this progressive internal semidiurnal tide of the Poincaré type propagating seaward, associated with the standing cross-channel wave. W. D. Forrester (unpublished data 1974) suggested that these internal tides are generated at the head of the Laurentian Channel, near the mouth of the Saguenay River.

Other observations of currents, temperature, and salinity made by Ingram (1975) confirmed the existence of the vertical displacements of large amplitude of the water masses in this region and ascribed them to the internal tides. He suggested that these internal tides are the mechanism responsible for the upwelling of the nutrient-rich deep water, at least during spring tides (fortnightly enrichment). In contrast, P. Greisman and R. G. Ingram (unpublished data) have found that during neap tides the nitrate enrichment of surface water results mainly from the seaward flow of the surface waters coming from further up the estuary.

In 1971 and 1972 we observed simultaneous tide-dependent variations of temperature, salinity, dissolved oxygen, nitrate, phosphate, and chlorophyll *a* in this region of the St. Lawrence estuary. They complement the findings of W. D. Forrester (unpublished data 1974) and Ingram (1975), based uniquely on physical data, and allow us to discuss the characteristics, generation, and propagation of the internal tides in the St. Lawrence estuary. Our observations were made during tides of similar height in 1972.

Materials and Methods

Eleven stations were sampled in the St. Lawrence estuary (Fig. 1). The sampling lasted 13 h at each station, with a periodicity of 1 or 2 h. Stations 063 and F172 were sampled on August 21 and 22, 1971, respectively. In 1972 all stations except F172 were sampled from June 28 to July 12. Stations 10, 063, 70, and 135 were sampled on June 28, 29, 30, and July 1, respectively; stations 277, 240, 190, and 170 were sampled on July 8 and 9, respectively, and stations 90, 063, and 220 on July 10, 11 and 12, respectively. On July 8 and 9 the periodicity of sampling was 2 h, which allowed the ship to travel from one station to the other to sample two stations in the same day. The sampling of stations F172, 063, 10, 135, and 220 was done while the ship was anchored. All the other stations were done adrift. The maximum drift was set at 0.5 km, after which the ship was brought back to the designated position. Because of the strong currents experienced during certain phases of the tide, the ship was repositioned frequently, sometimes as often as 3 times during a sampling period of 45 min.

Samples were taken in Niskin or Van Dorn bottles at the following depths: 0, 5, 10, 15, 25, 50, 100, and 150 m (station depth permitted). The first subsample collected from each depth was used immediately for determination of dissolved oxygen following the method of Winkler as modified by Strickland and Parsons (1968). A second subsample was filtered immediately through a GF/C glass filter for later determination of chlorophyll *a*. The third and fourth subsamples were also filtered through GF/C paper before being frozen at −15 C for later analysis of phosphate and nitrate. A fifth subsample was drawn for salinity determination. The temperatures were obtained with bathythermographs at each station. In 1971 we also used Nansen reversing bottles.

Analysis for phosphate, nitrate, and chlorophyll *a* was made according to the methods described in Strickland and Parsons (1968). Salinity samples were analyzed by the inductive conductivity method using a Hytech Model 6220 salinometer.

Results

OBSERVATIONS OF 1971

Figure 2 shows tide-dependent variations of temperature, salinity, dissolved oxygen, phosphate, nitrate, and chlorophyll *a* at station 063 of the St. Lawrence estuary on August 21, 1971. One can easily recognize the periodic appearance and disappearance of an intermediate, cold-water

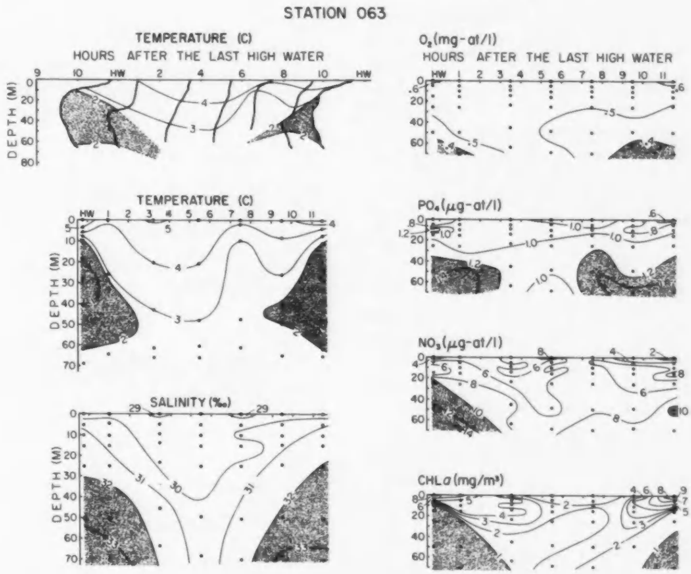

FIG. 2. Tide-dependent variations of temperature, salinity, dissolved oxygen, phosphate, nitrate, and chlorophyll *a* at station 063 of the St. Lawrence estuary in 1971. Temperature in the left upper part, from B.T. (bathythermographs); in the middle part, from reversing thermometers.

layer characterized by the 1 and 2 C isotherms and the 32 and 33‰ isohalines. This layer is not noticeable at low water but is observed at 8 m below the surface at high water. It is also possible to note the appearance and disappearance of a deep water mass with chemical characteristics similar to those of the deep water of the St. Lawrence channel: low oxygen (0.35–0.45 mg-at/liter) and high phosphate (1.2–1.4 μg-at/liter) and nitrate (10–14 μg-at/liter) concentrations. The chlorophyll *a* is also much less abundant (less than 1 mg/m³). Briefly, Fig. 2 illustrates clearly the existence of considerable tide-dependent vertical oscillations of the various water masses at station 063 on August 21, 1971.

Similar observations carried out the next day at station F172 (Fig. 3) have shown that the surface waters at this station are relatively warmer and less saline than those at station 063. Vertical oscillations of some isotherms, isohalines, and isopleths of dissolved oxygen, phosphate, nitrate, and chlorophyll *a* can also be seen deeper in the water column. These vertical oscillations are, however, of much smaller amplitude than at station 063 (15–20 m at station F172, compared with 45–50 m at station 063 by reference to the 3 C isotherm). The appearance of the high-nutrient and low-oxygen concentrations in the deep waters at station F172 coincides fairly well with the high excursion of intermediate waters at station 063.

OBSERVATIONS OF 1972

Stratification — The three typical water layers in the Laurentian Channel (Lauzier and Bailey 1957) are easily recognized from the various bathythermograph measurements made at different phases of the tide in 1972 (Fig. 4–5). The surface layer is characterized by relatively warmer waters (2–6 C) and its thickness varies according to the state of the tide. Likewise, the 2 C isotherm represents the upper and lower limit of a cold intermediate layer, the thickness and the depth of which depend similarly on the tidal phase. This layer was very cold and thick in 1972, compared with the same period in 1971 (Therriault 1973). It contains a large central core with temperature < -1 C. The surface layer and the cold layer are separated by a thermocline the depth of which varies according to the geographical location of the station and the time of observation. The deep layer has temperatures exceeding 2 C and is seen only at the deeper stations (90, 170, 190, 240, 277).

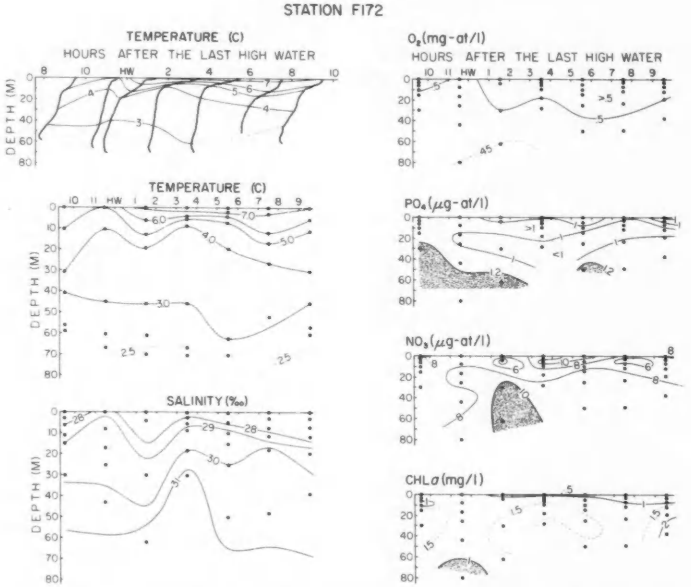

FIG. 3. Tide-dependent variations of temperature, salinity, dissolved oxygen, phosphate, nitrate, and chlorophyll *a* at station F172 of the St. Lawrence estuary in 1971. Temperature in the left upper part, from B.T.; in the middle part, from reversing thermometers.

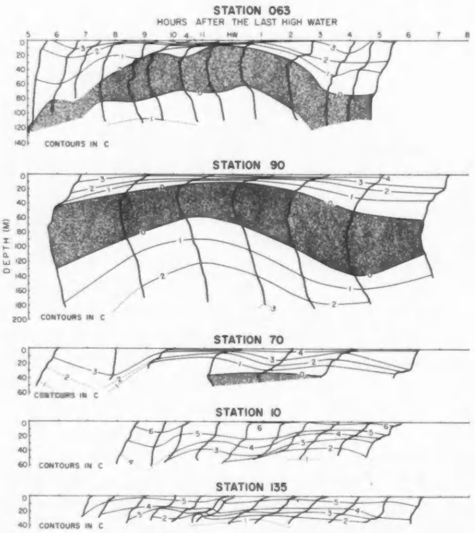

FIG. 4. Tide-dependent variations of the vertical profile of temperature at station 063, 90, 70, and 10 of the St. Lawrence estuary in 1972. Gray bands bounded by 0 C isopleths indicate water colder than 0 C.

On the other hand, if we look at the vertical distribution of the densities (σ_T), only two distinct layers are really recognized at the deeper stations in the estuary. The first layer extends from the surface to the lower limit of the intermediate cold layer and shows a regular increase of σ_T with depth. The second layer extends from the cold layer to the bottom and is characterized by a much smaller increase of σ_T with depth. There exists a very distinct interface between these two layers and its depth is variable with the tide. Figure 6 presents a typical example of the vertical distribution of σ_T.

Figures 7 and 8 describe the tide-dependent variations of dissolved oxygen, nutrient, and chlorophyll *a* at the various stations in the estuary. These figures illustrate clearly the decreasing concentration of dissolved oxygen from the surface to the bottom. By considering the percent O_2 saturation one can differentiate three layers: 1) the surface layer (0–10 m), always saturated; 2) an intermediate layer, essentially the cold layer with saturation values of 80–95%; 3) the deep layer, not so well oxygenated with saturation values varying from 80% just below the cold layer to <50% at 150 m. The relative depth and thickness of these three different layers vary with tide.

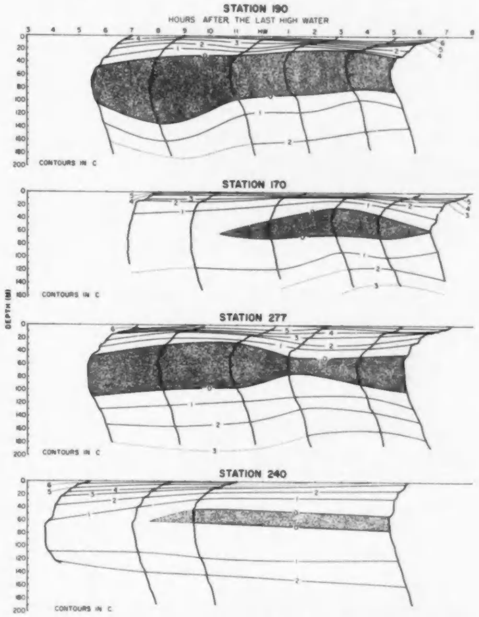

FIG. 5. Tide-dependent variations of the vertical profile of temperature at station 190, 170, 135, 277, and 240 of the St. Lawrence estuary in 1972. Gray bands bounded by 0 C isopleths indicate water colder than 0 C.

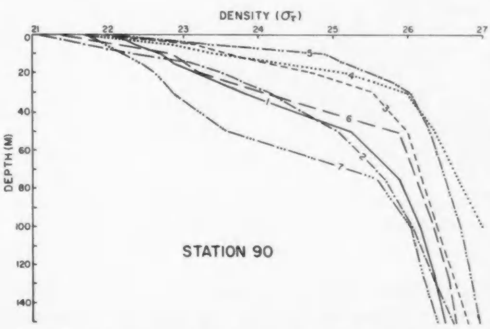

FIG. 6. Tide-dependent variations of density (σ) with depth, at station 90 of the St. Lawrence estuary in 1972. (1) 1 h, (2) 1 h 25 min, (3) 3 h 18 min, (4) 5 h 24 min after low tide, and (5) 1 h 30 min, (6) 3 h 20 min, (7) 5 h after high tide.

The vertical distributions of the nutrients follow, generally, the distribution of the densities. The concentrations are relatively lower in the upper layer: 0.6–1/μg-at PO_4/liter and 6–12 μg-at NO_3/liter. Those of the lower layer are much higher (1.2–2.0 μg-at PO_4/liter and 12–16 μg-at NO_3/liter.

The observed chlorophyll *a* concentrations were very low during our sampling period in 1972. The maximum observed was 0.5 mg/m^3, at 5 m at station 277. Generally, the vertical distribution of the chlorophyll presents a decreasing gradient with depth; the minimal values are rapidly reached.

Vertical oscillations — A comparative look at Fig. 3–10 distinctly reveals the existence of cyclic tide-dependent vertical oscillations of the various isotherms and isopleths at all stations. These oscillations are readily apparent from the upward movement of the cold intermediate and the deep layers. There is almost perfect correspondence between the vertical oscillations of the different isotherms and the oscillations of the less concentrated isopleths of dissolved oxygen and chlorophyll *a* and the more concentrated isopleths of phosphate and nitrate.

Ascribing these cyclic oscillations to waves, we note that their amplitude is different according to the geographical location of the station. Thus, referring to the upper 0 C isotherm, the amplitude is 120 m at station 063, 45 m at station 90, 25 m at station 190, etc. Therefore, the amplitude of the wave is higher at the western stations (063, 90) than at the eastern stations (277, 240) and at the northern stations (90, 190, 277) than at the southern stations (70, 170). The period of this wave coincides with that of the semidiurnal tide. The crest of the wave is located at high water (between the 5th h after low water and the 1st h after high water) at stations 063, 70 and 90; at the beginning of the ebb tide (between the 1st and the 4th h after high water) at stations 10, 135, 170 and 190; and at the end of the flood tide (between the 4th and the 6th h after low tide) at stations 240 and 277. On the other hand, the trough of the wave is located at low water at stations 063, 70, and 90; at the beginning of the flood tide at stations 10, 135, 170, and 190; and at the end of the flood tide at station 277. Thus, the appearance of the crest of the wave at the various stations in the estuary is shifted in phase from the west to the east.

Finally, we emphasize the periodic appearance and disappearance of the 0 C isotherm at stations 240, 170, and 70 located along the south margin of the Laurentian Channel. A similar phenomenon is present at stations 063, 10, and 135 for the upper isotherms of 1, 2, and 3 C and for the lower isotherms of 2 and 3 C at stations 90 and 170. The periodic appearance of these isotherms occurs during the formation of the crest of the wave at each station. Isopleths of lower concentrations of dissolved oxygen and higher values of phosphate and nitrate are associated with these various isotherms.

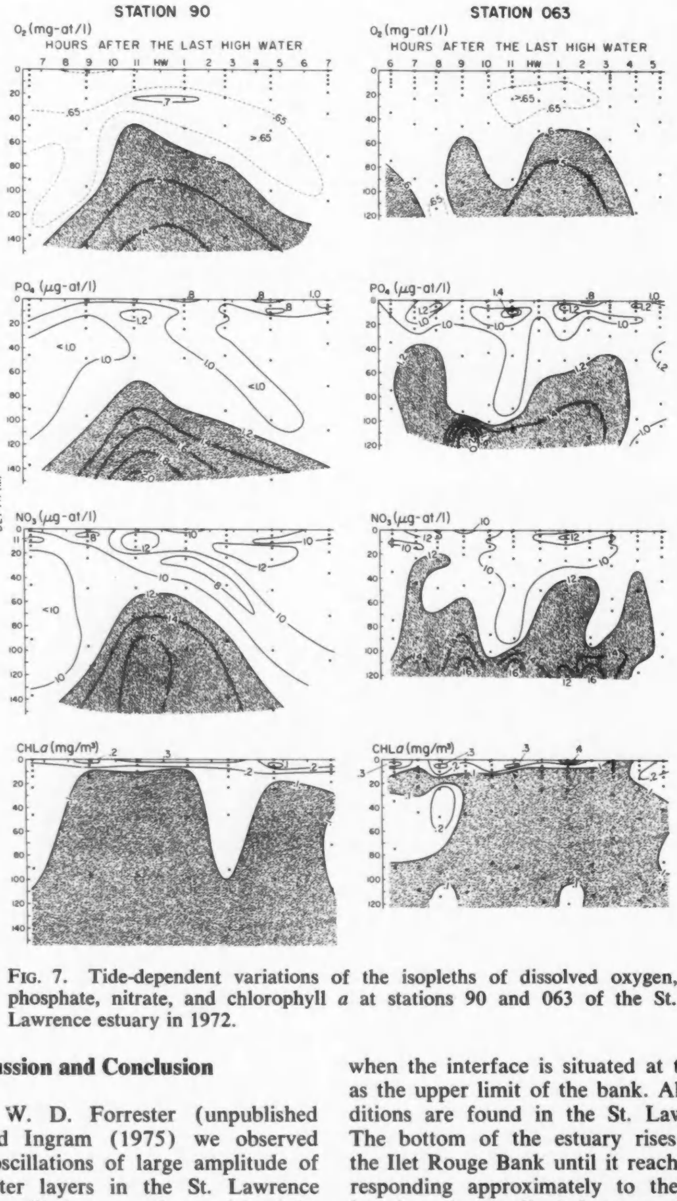

FIG. 7. Tide-dependent variations of the isopleths of dissolved oxygen, phosphate, nitrate, and chlorophyll *a* at stations 90 and 063 of the St. Lawrence estuary in 1972.

Discussion and Conclusion

Similarly to W. D. Forrester (unpublished data 1974) and Ingram (1975) we observed cyclic vertical oscillations of large amplitude of the various water layers in the St. Lawrence estuary. These oscillations can be explained by the presence of internal tides of the same period as the semidiurnal tide in the estuary.

In a stratified water column, an irregularity in the bottom configuration can generate internal tides (Defant 1961; Proudman 1953; Lafond 1962). Also, internal tides can be generated when a tidal current flows over a shallow bank and when the interface is situated at the same depth as the upper limit of the bank. All of these conditions are found in the St. Lawrence estuary. The bottom of the estuary rises abruptly near the Ilet Rouge Bank until it reaches a depth corresponding approximately to the depth of the interface (pycnocline) between the surface and the deep layer. One can speculate that the surface tidal wave, in flowing over this submarine barrier, produces an internal tide of large amplitude that propagates in the seaward direction. Our observations showed that the amplitude of the vertical displacement is maximum at station 063, located over the abrupt rising bottom at the head

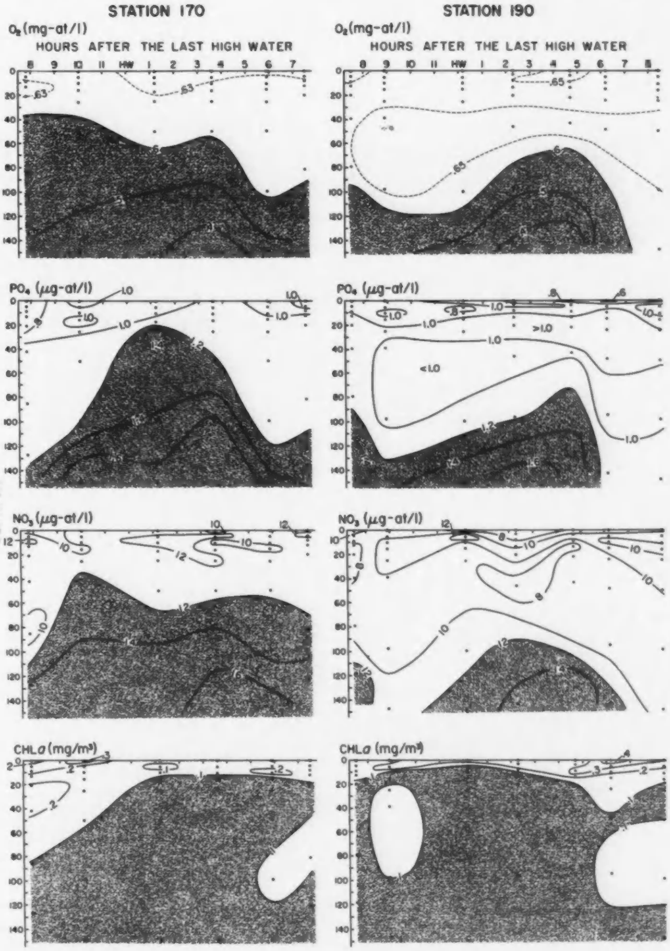

FIG. 8. Tide-dependent variations of the isopleths of dissolved oxygen, phosphate, nitrate, and chlorophyll *a* at stations 170 and 190 of the St. Lawrence estuary in 1972.

of the Laurentian Channel. Furthermore, the crest of the internal wave occurs during high water at this site. Consequently, it can be argued that the internal tide is generated in this area.

By following the horizontal displacement of the crest of the wave, we can characterize its propagation. The area of the estuary above the Saguenay appears to be less affected by this internal tide, if we judge by the differences in amplitude of the vertical oscillations between station 063 and F172 in 1971. Thus, after generation, the internal tide propagates longitudinally (mainly) in the seaward direction, as suggested by the sequential appearance of the crest in the west–east direction and by its decreasing amplitude in the same direction. Since the crest of the wave is observed at approximately the same tidal phase at the stations 063, 277, and 240, we can estimate its wavelength to be around 40 km.

There is no fundamental discrepancy between this value and the value of 60 km established by Forrester (1974), since the characteristics of this internal tide can vary according to the intensity of the stratification, the relative depth of the interface, and the height of the surface tide.

W. D. Forrester (unpublished data 1974) showed the presence of a transverse standing wave associated with the longitudinal wave. Our

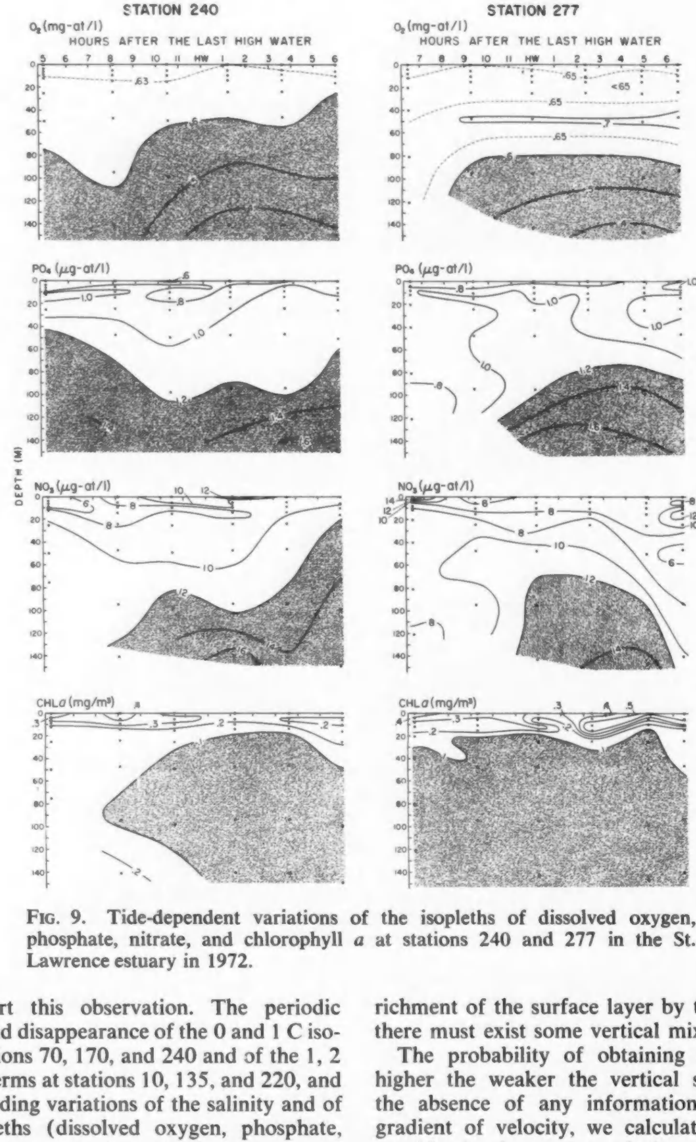

FIG. 9. Tide-dependent variations of the isopleths of dissolved oxygen, phosphate, nitrate, and chlorophyll *a* at stations 240 and 277 in the St. Lawrence estuary in 1972.

results support this observation. The periodic appearance and disappearance of the 0 and 1 C isotherms at stations 70, 170, and 240 and of the 1, 2 and 3 C isotherms at stations 10, 135, and 220, and the corresponding variations of the salinity and of various isopleths (dissolved oxygen, phosphate, nitrate) suggest a north–south horizontal displacement of the water masses accompanying the propagation of the crest of the longitudinal internal tide.

When an internal tide is traveling in a water column, the various water layers oscillate vertically but remain generally unchanged in their characteristics. The water ascending to the surface during one phase of the tidal cycle must go down in the other phase. To have nutrient enrichment of the surface layer by the deep waters, there must exist some vertical mixing.

The probability of obtaining such mixing is higher the weaker the vertical stratification. In the absence of any information about vertical gradient of velocity, we calculated an index of stratification based on vertical gradients of density to estimate this probability. This index is defined as

$$m = \frac{Z_{i+1} - Z_i}{C_{i+1} - C_i}$$

where m is the reciprocal of the rate of density change with depth (stratification); Z_i is the depth in meters; and C_i is the value of σ_T at the depth

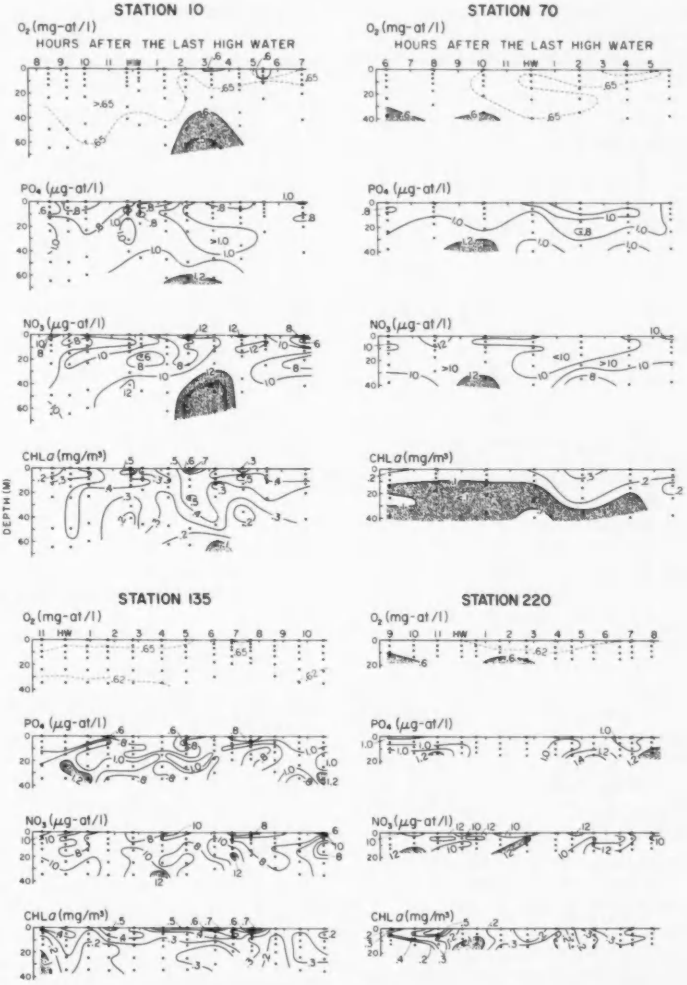

FIG. 10. Tide-dependent variations of the isopleths of dissolved oxygen, phosphate, nitrate, and chlorophyll *a* at stations 10, 70, 135, and 220 of the St. Lawrence estuary in 1972.

Z_i. The higher the index, the higher the probability of obtaining vertical mixing. Indeed, when $m \rightarrow \infty$, the water layer is unstratified and presents ideal conditions for vertical mixing. The indices averaged over a complete tidal cycle are presented in Table 1 for each station of the estuary in 1972. At first sight, the index of stratification is found to increase with depth. But a more careful examination shows that we can always divide the water column in two distinct layers: 1) the upper 50 m, with lower values and 2) the deep waters, with much higher values. This 50-m limit corresponds to the mean depth of the pynocline in the St. Lawrence estuary (Fig. 6). These results suggest the existence, vertically, of two different layers characterized by different rates of vertical mixing.

Spatially, we expect that the enrichment zones are the regions where the surface waters exhibit the higher *m* values. If we average the index of stratification of the upper 30 m for each station in the estuary (Table 1), it is possible to situate the higher indices in the region of the Ilet Rouge Bank, at the head of the Laurentian Channel

TABLE 1. Vertical and horizontal variations of the index of stratification in the St. Lawrence estuary in 1972 (see text for explanations).

Depth interval (*m*)	Stations									
	277	240	220	190	170	135	90	70	10	063
0–5	31	7	72	9	50	14	9	19	7	20
5–10	5	7	10	9	4	23	11	16	11	20
10–15	7	5	12	8	6	10	18	55	12	15
15–20	10	7		9	9	30	16	40	15	20
20–25	14	10		12	14	59	18	24	20	25
25–30	12	15		13	11	11	19	21	13	25
30–50	15	21		33	31		35		14	38
50–70	62	54		77	122		78			211
75–100	94	93		93	115		92			137
100–125	121	126		138	153		144			
125–150	155	126		137	155					
Tidal avg of top 30 m or less	13	9	31	9	16	25	15	29	13	21

(Fig. 11). This figure shows clearly the deviation of the various isolines of stratification toward the south shore of the estuary. A similar pattern has been described many times in the literature (Dugal 1934; Gaudry 1938; Nadeau 1938; etc.) and is thought to be associated with the deviation of the outflowing surface water of the estuary by the outward surface current of the Saguenay fjord. The regions exhibiting the higher indices of mixing appear to be associated mainly with the outflowing surface waters of the estuary. However, current studies made recently by R. G. Ingram (unpublished data) have shown that this region of the Ilet Rouge Bank is effectively a site of an intense vertical mixing. The suggestion that nutrients are introduced into the surface layers by mixing (Ingram 1975) is then tenable.

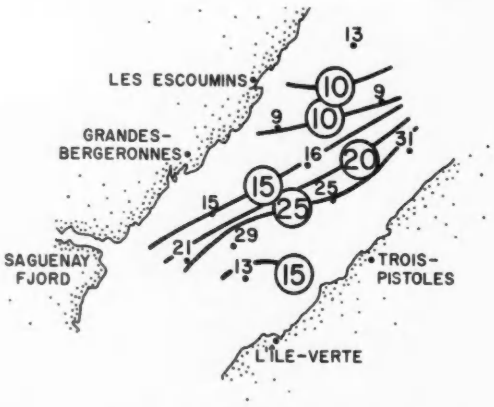

FIG. 11. Spatial variations of the averaged index of stratification (upper 30 m) in the water column of the St. Lawrence estuary in 1972.

Considering the hourly average values (top 30 m) at stations 063, 70, 135, and 220 (showing the higher values on Fig. 11), we notice the existence of two discrete maxima at the first three stations. Only one maximum is apparent at station 220. Hourly averaged values of 28, 45, and 142 are found at stations 063 and 70 at high water and at station 135, 4 h after low water, respectively. These correspond with the time of appearance of the crest of the internal tide at these stations. Great care must be taken when interpreting these results. The presence of these maxima associated with the crest of the internal tide does not necessarily mean real mixing between surface and intermediate waters, but is more likely linked with higher values of deeper waters (as deep as 100 m) present above the 30 m averaging depth limit used. Since the influence of the internal tide is not felt at the very shallow station 220, we do not notice a distinct maximum at this station at the time of the appearance of the crest. On the other hand, another maximum is also noticed at these four stations (48 at 063, 84 at 70, 139 at 135, and 183 at 220) from 4 to 6 h after the crest of the internal tide. These maxima are associated with the outward flow of the surface waters of the estuary. Also, at this particular phase of the tide the higher nutrient concentrations are found in the surface waters.

The penetration of intermediate waters (high-nutrient and low-oxygen concentrations) beyond the Laurentian Channel at the time of the internal tide crest is shown clearly by the Fig. 2 and 3.

This upstream penetration of nutrient rich waters could be quite extensive as suggested by the presence of arctic and subarctic copepods at about 100 km beyond Ilet Rouge (Bousfield et al. 1975). This upstream region of the estuary is thought to be one of very intense turbulent mixing of the whole water column (P. Greisman and R. G. Ingram unpublished data). The nutrient-rich waters that have penetrated beyond the Laurentian Channel are then introduced into the surface layer by vertical mixing before flowing downstream to enrich the surface waters of the estuary. Our observation of maximum values of the index of mixing at low tide at stations 063, 70, 135, and 220 support this view. There is no reason to reject Steven's (1974) "nutrient pump" hypothesis that the nutrient enrichment of the surface waters of the estuary originates mainly from phenomena taking place in the region of the mouth of the Saguenay fjord. However, the mechanism of the "nutrient pump" is not simply tide-induced upwelling. Penetration of intermediate waters in the upstream region associated with intense vertical mixing in this region may prove to be a much more efficient mechanism.

Acknowledgments

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BOUSFIELD, E. L., G. FILTEAU, M. O'NEILL, AND P. GENTES. 1975. Population dynamics of zooplankton in the middle St. Lawrence estuary, p. 325–351. *In* L. E. Cronin [ed.] Estuarine research. Vol. 1. Academic Press, Inc., New York, N.Y. 738 p.

CANADIAN HYDROGRAPHIC SERVICE. 1939. Tidal current charts. St. Lawrence estuary, Orleans Island to Father Point, Ottawa, Canada. Dep. Environ., Fish. Mar. Serv., Can. Hydrogr. Serv.

DEFANT, A. 1961. Physical oceanography. Vol. II. Pergamon Press, Inc., New York, N.Y. 598 p.

DUGAL, L.-P. 1934. Observations sur le chlore total et l'oxygène dissous de l'estuaire du Saint-Laurent, saisons 1932–1933. Nat. Can. 62: 165–181.

FORRESTER, W. D. 1974. Internal tides in the St. Lawrence estuary. J. Mar. Res. 32: 55–66.

GAUDRY, R. 1938. Les températures de l'estuaire du Saint-Laurent. Contrib. Stn. Biol. Saint-Laurent, Univ. Laval 13: 1–24.

HUNTSMAN, A. G. 1923. The influence of tidal oscillation on vertical circulation in estuaries. Trans. R. Soc. Can. Ser. 3 17: 11–14.

INGRAM, R. G. 1975. Influence of tidal-induced vertical mixing on primary productivity in the St. Lawrence estuary. Mem. Soc. R. Sci. Liege 6: 59–74.

LAFOND, E. C. 1962. Internal waves. Part I. p. 731–751. *In* M. N. Hill [ed.] The sea. vol. 1. (Physical oceanography). Intersciences Publishers, Inc., New York, N.Y. 864 p.

LAUZIER, L., AND W. B. BAILEY. 1957. Features of the deeper waters of the Gulf of St. Lawrence. Bull. Fish. Res. Board Can. 111: 213–250.

NADEAU, A. 1938. Salinité des eaux de l'estuaire du Saint-Laurent. Contrib. Biol. Stn. Saint-Laurent, Univ. Laval 11: 1–19.

PROUDMAN, J. 1953. Dynamical oceanography. Methuen and Co., Ltd., London. 409 p.

STEVEN, D. M. 1974. Primary and secondary production in the Gulf of St. Lawrence. Mar. Sci. Cent., McGill Univ., Montreal, Que. MS Rep. 26: 116 p.

STRICKLAND, J. D. H., AND T. R. PARSONS. 1968. A manual of seawater analysis. 2nd (revised) ed. Bull. Fish. Res. Board Can. 125: 1–203.

THERRIAULT, J. C. 1973. Variations des propriétés physico-chimiques et biologiques d'une zone de mélange de de l'estuaire du Saint-Laurent. M.Sc. Thesis. Laval Univ., Que. 155 p.

VACHON, A., R. GAUDRY, AND R. BERNARD. 1938. Contribution à l'étude des phosphates dans l'estuaire du Saint-Laurent. Contrib. Stn. Biol. Saint-Laurent, Univ. Laval 12: 1–11.

Marine Phytoplankton Growth in High Concentrations of Pulpmill Effluent

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STOCKNER, J. G., AND A. C. COSTELLA. 1976. Marine phytoplankton growth in high concentrations of pulpmill effluent. J. Fish. Res. Board Can. 33: 2758–2765.

Axenic culture studies with the marine phytoplankton *Skeletonema costatum*, *Dunaliella tertiolecta*, and *Amphidinium carteri* demonstrated the ability of these species to adapt to and exhibit normal growth in relatively high concentrations of pulpmill effluent. *Skeletonema costatum* and *A. carteri* required a preadaptation period prior to commencement of exponential growth in high concentrations of kraft effluent (20–30%), while *D. tertiolecta* exhibited exponential growth in 90% kraft effluent with no requirement for preadaptation. Of six pulpmill effluent types tested, kraft was considered most inhibitory to growth, while combined kraft and newsprint effluent passed through an XAD-8 resin column was least inhibitory. The effects of the six effluent types on lag, exponential, and stationary growth phases are discussed in relation to in situ concentrations and relative ecological significance in the coastal marine environment.

STOCKNER, J. G., AND A. C. COSTELLA. 1976. Marine phytoplankton growth in high concentrations of pulpmill effluent. J. Fish. Res. Board Can. 33: 2758–2765.

La culture axénique des phytoplanctontes marins *Skeletonema costatum*, *Dunaliella tertiolecta* et *Amphidinium carteri* démontre l'aptitude de ces espèces à s'adapter à des concentrations relativement élevées d'effluent de moulin de pâte à papier et à y poursuivre une croissance normale. *Skeletonema costatum* et *A. carteri* exigent une période de préadaptation avant le début de la croissance exponentielle dans des hautes concentrations d'effluent de papeterie kraft (20–30%), alors que *D. tertiolecta* montre une croissance exponentielle dans une concentration de 90% d'effluent de papeterie kraft, sans période de préadaptation. Des six types d'effluents de moulins de pâte à papier, l'effluent de papeterie kraft est celui qui inhibe le plus la croissance, alors que l'effluent combiné de papeterie kraft et d'usine de papier à journal ayant traversé une colonne de résine XAD-8 a le moins d'effet. Nous discutons des effets des six types d'effluents sur les phases de retard, exponentielles et stationnaires de croissance en relation avec les concentrations in situ et de leur signification écologique relative dans l'environnement marin côtier.

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ENVIRONMENTAL effects of pulpmill effluent discharge on marine phytoplankton communities in British Columbia coastal waters are not well understood. Tully (1949), Waldichuk (1954), and Parker and Sibert (1972) have studied effects of kraft mill effluent discharge in Alberni Inlet, B.C. as it relates to depressed oxygen levels. Recently, Stockner et al. (1975) and Parker and Sibert (1976) have documented field effects of light attenuation by pulpmill effluent stain on marine phytoplankton photosynthesis, but little is known of potential toxicity (inhibitory potential). This laboratory study was aimed directly at the question of pulpmill effluent toxicity and was designed to answer specific questions concerning toxicity raised by previous field investigations.

The use of axenic phytoplankton cultures of three species, representing three major taxonomic groups, permitted observation of growth free of possible synergistic effects by other organisms, especially bacteria. The possibility of phytoplankton adaptation to effluent was also considered in this investigation. To distinguish light limitation effects from the pollutant toxicity or growth-inhibiting effects, we chose incubator light intensities high enough to overcome the light attenuation caused by pollutant color. We also ensured that sufficient nutrients and stable pH conditions were present to offset possible growth limitation from these factors. These precautions enabled us

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to establish effects of pulpmill effluent free of the many uncontrollable physical, chemical, and biological parameters prevalent in the marine environment.

Materials and Methods

Bioassays were conducted in a seawater medium of composition shown in Table 1. The medium was high in silicate and designed to support heavy diatom populations. The medium was stored in a 6-liter flask in a cold room with no illumination prior to use. After adding the required amounts of medium and filtered sea water, 125 ml screw-cap flasks were autoclaved for 20 min at 120 C, cooled, and stored until ready for use. Upon termination of each experiment, all flasks were cleaned in hot water and air dried.

Kraft and newsprint effluents were collected October 31, 1975 from Crofton Pulp and Paper Mill, British Columbia Forest Products Ltd., Crofton, B.C. At the time of effluent collection, the mill was pulping primarily western hemlock (*Tsuga heterophyla*), and samples collected were considered "typical" of most coastal British Columbia mills lacking secondary treatment facilities (biobasin). The effluent from newsprint and kraft processes is blended and then discharged without treatment to the marine receiving waters. All effluents were stored in 20-liter, plastic carboys in a cold, dark room prior to use.

Several steps were required to prepare the effluent for use. Initially, some of the kraft and newsprint effluents were combined in a 50:50 blend. The three effluents (kraft, newsprint, and blend) were then filtered through Whatman GF/C glass fiber filters to remove wood fibers and debris. A portion of the blend was passed through an Amberlite XAD-8 resin column (Rohm and Haas, Canada Ltd.) to remove color. This procedure added another effluent for testing, giving a total of four basic effluent types (kraft, newsprint, blend, and single-pass).

Since pulpmill wastes are diluted with fresh water, all effluents were adjusted to a salinity of 20‰ by adding 5 g NaCl for every 50 ml effluent. The pH of each effluent was adjusted with aqueous 0.1 N HCl to 7.6–7.8.

Effluents were filtered on preweighed Whatman GF/C glass fiber filters, dried to constant weight at 105 C, weighed, and ashed in a muffle furnace at 500 C for 4 h to determine loss on ignition (% LOI = organic) and particulate organic and inorganic content. Organic and inorganic carbon content of the four effluent types were determined by the Pacific Region, Water Quality Branch Chemical Laboratory, Department of the Environment, North Vancouver.

The effluents were analyzed for nitrate (NO_3), nitrite (NO_2), ammonia (NH_3), and total phosphate (PO_4) by the Fisheries Operations/Environmental Protection Service analytical laboratories, Pacific Environment Institute, West Vancouver. Methods used are as outlined in their manual (Fisheries/EPS 1974).

An extract of blend effluent was analyzed by electron-capture gas chromatography (flame ionization detector), liquid chromatography, and sodium fusion to determine the presence and amount of chlorinated compounds, fatty and resin acids, lignin derivatives, and neutral compounds.

Table 1. Composition of phytoplankton growth media.

	(mg)	(μmole)
KNO_3	50	(500)
$NaH_2PO_4 \cdot H_2O$	6.9	(50)
$Na_2SiO_3 \cdot 9H_2O$	140	(500)
Vitamins	(μg)	
Thiamine · HCl	500	(1.48)
Biotin	1	(0.0041)
B_{12}	1	(0.00134)
Trace-metal ions (chelated)[a]	(mg)	
$Na_2EDTA \cdot 2H_2O$	8.1	(21.8)
$FeCl_3 \cdot 6H_2O$	2.7	(10.0)
$MnSo_4 \cdot 4H_2O$	1.125	(5.0)
$ZnSO_4 \cdot 7H_2O$	0.575	(2.0)
$Na_2MoO_4 \cdot 2H_2O$	0.243	(1.0)
$CuSO_4 \cdot 5H_2O$	0.025	(0.1)
$CoSO_4 \cdot 7H_2O$	0.014	(0.05)
Buffer		
Tris · HCl	1 g	(8.3 mmole)
Distilled water	200 ml	
Sea water, Strait of Georgia approx. 30‰ (range 25–30‰)	to 1 liter	

[a] pH of stock solutions of these components was adjusted to 7.5–7.6 with aqueous HCl or NaOH before incorporation with the rest of the media.

After passage of blend effluent through an Amberlite XAD-8 resin column, color was stripped from the resin with methanol. Liquid–liquid extraction produced two additional fractions of effluent, a diethylether soluble fraction (almost colorless) and a diethylether insoluble fraction (colored). Gas chromatographic analysis was done only on the soluble fraction.

Apparent and true color values were determined for kraft, blend, single-pass, and the two diethylether fractions using APHA et al. (1971) platinum–cobalt color standards. Apparent color was determined before filtration and true color after filtration through Whatman GF/C glass fiber filters.

Three species in axenic culture were selected as representatives of three dominant marine taxa, common in coastal waters: *Skeletonema costatum* (Chrysophyta), *Dunaliella tertiolecta* (Chlorophyta), and *Amphidinium carteri* (Pyrrophyta) were used in all experiments.[1]

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[1] *Skeletonema costatum*: clone *Skel* from culture collection of Woods Hole Oceanographic Institution; isolated by R. R. L. Guillard from Long Island Sound, New York. *Dunaliella tertiolecta*: clone *Dun* from culture collection of Woods Hole Oceanographic Institution; isolation details unknown. *Amphidinium carteri*: clone *Amphi 1* from culture collection of Woods Hole Oceanographic Institution; isolated by R. R. L. Guillard from Falmouth Great Pond, Massachusetts.

TABLE 2. Chemical analysis of effluents tested in this investigation.

	Kraft	Newsprint	Blend	Single-pass	Diethylether insoluble	Diethylether soluble
Dry seston ($mg \cdot m^{-3}$)	201	72	141	—	—	—
Ash wt ($mg \cdot mg^{-3}$)	8.0	8.5	12.0	—	—	—
% Loss on ignition	96.0	88.2	91.5	—	—	—
Organic C ($mg \cdot liter^{-1}$)	210	260	240	98	—	—
Inorganic C ($mg \cdot liter^{-1}$)	8.1	10.5	9.3[a]	2.8	—	—
NO_3-N ($mg \cdot liter^{-1}$)	0.04	0.01	0.03	0.08	—	—
NO_2-N ($mg \cdot liter^{-1}$)	0.03	0.03	0.02	0.01	—	—
NH_3-N ($mg \cdot liter^{-1}$)	1.00	0.18	0.52	0.45	—	—
TPO_4-P ($mg \cdot liter^{-1}$)	0.30	2.60	1.45[a]	1.00	—	—
Apparent color	2160	—	1600	360	1344	45
True color	1680	—	1400	320	880	37

[a] Interpolated value.

Prior to every experiment a Cary recording spectrophotometer (Model 15), an Orion digital pH meter (Model 801), and an Auto-lab salinometer (Model 601) were used to analyze each concentration of effluent for color, pH, and salinity, respectively. Control flasks consisted of autoclaved filtered sea water at the same pH and salinity. Test flasks were incubated in duplicate with one control flask for each effluent concentration. There was a total volume of 50 ml in each flask, with effluent concentration expressed as a percentage of total volume.

All effluent tested was filtered through a sterile, enclosed 0.20 μm plain membrane filter unit (Nalgene 120 type) to remove bacteria. One ml of axenic phytoplankton stock culture (7–21 days old), plus 2 drops of total vitamin mix, was added to each flask containing effluent and media, using standard aseptic techniques. Flasks were incubated stationary in a Sherer Laboratory Incubator in continuous, cool-white fluorescent light at 15 C and illumination intensity of 6500 lx. Population growth was monitored by daily cell counts using a Spencer Bright-Line hemacytometer. To count motile *D. tertiolecta* and *A. carteri* cells, one drop of 3% Formalin was added to each aliquot of culture and the number of cells per milliliter corrected for dilution. Cultures were incubated until stationary growth was attained, which, depending on species and effluent concentration used, ranged from 9 to 35 days.

When the cultures reached quiescence (stationary phase), they were terminated by filtering onto a Whatman GF/C glass fiber filter for chlorophyll *a* analysis. Filters were macerated in a tissue grinder with 10 ml of 90% acetone, and the filtrate analyzed for chlorophyll *a* on a Cary recording spectrophotometer (Model 15). The equation of Strickland and Parsons (1968) was used to calculate chlorophyll *a* in milligrams of chlorophyll *a* per cubic meter.

The doubling time of each unialgal population was computed using the formula:

$$D = \frac{\ln 2}{\ln C(t)/Co} \times t$$

where: D = doubling time in days (× 24 = hours); C(t) = concentration of phytoplankton at end of exponential phase of growth; Co = concentration of phytoplankton at beginning of exponential phase of growth; t = time in days between beginning and end of exponential phase of growth.

Results

The effluents tested were chemically different, but variation among effluents for given constituents was not large (Table 2).

Kraft effluent was most inhibitory to growth of *S. costatum* (experiments 1–6, Fig. 1A). After a prolonged lag period of 15 and 25 days, exponential growth began in 20% and in one of the duplicate flasks at 30% kraft. *Skeletonema costatum* in 40% kraft showed no growth. Newsprint effluent was less inhibitory than kraft with no lag at 10, 20, or 30%; however, as with kraft, there was no growth at 40% newsprint (Fig. 1B). The blend effluent affected growth only at higher concentrations (Fig. 1C), while with single-pass effluent there was no effect (Fig. 1D). Growth in the diethylether soluble or insoluble fractions showed reduced yield at 30 and 40% (Fig. 1E and 1F). Doubling times did not vary significantly among concentrations but chlorophyll *a* values showed a marked decrease with increasing concentrations of all effluents (Table 3).

In experiments 7–10, a short lag phase of 1–3 days was evident at only the highest concentrations of kraft and newsprint effluent; other effluents had no effect on growth of *D. tertiolecta* (Fig. 2). Doubling time of *D. tertiolecta* increased with increasing effluent concentration, and chlorophyll *a* showed an inverse relation to effluent concentration, being higher in test than in control flasks (Table 3).

The dinoflagellate *A. carteri* required a 15- and 17-day preadaptation period in 30 and 40% kraft effluent, respectively (experiments 11–14, Fig.

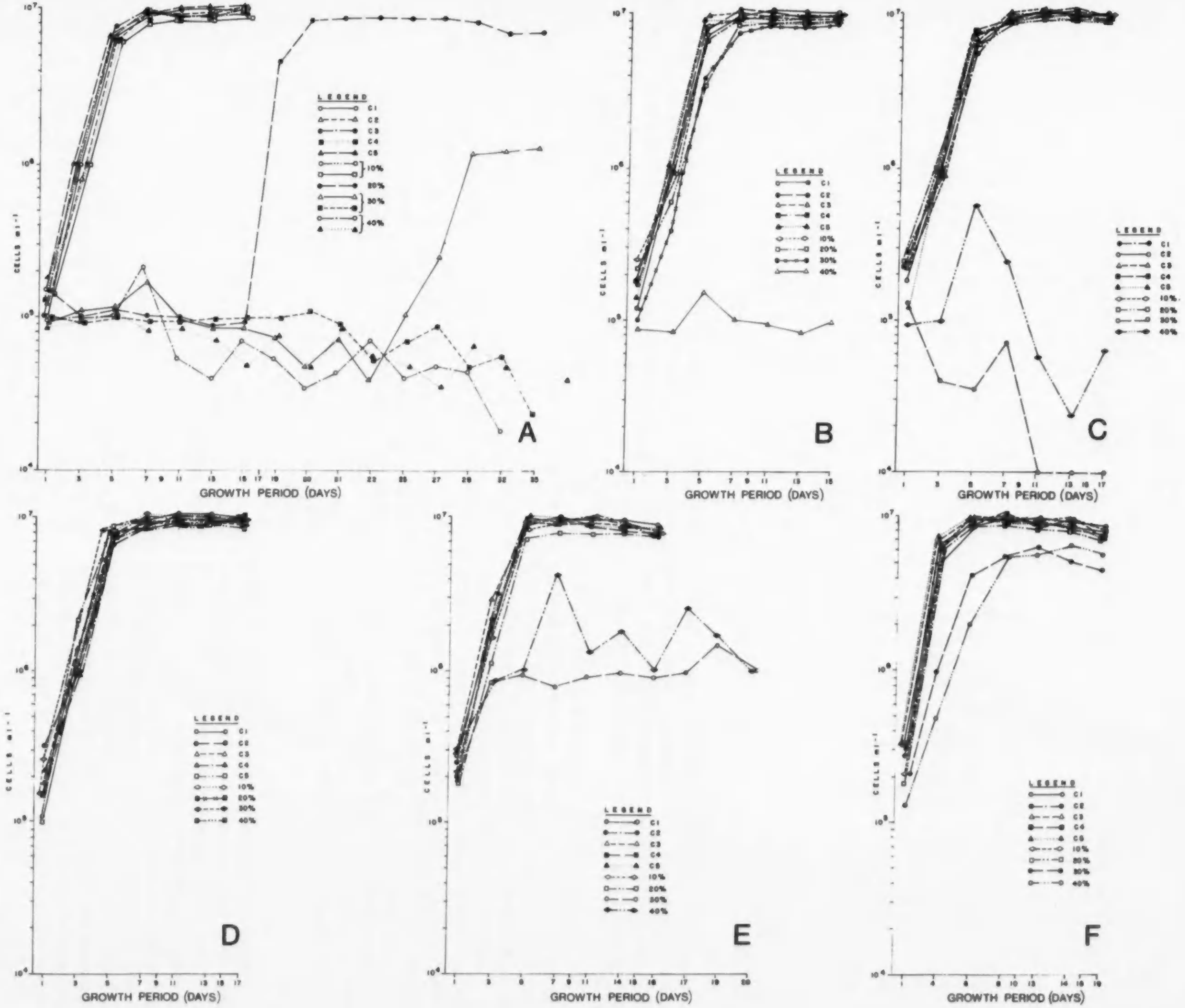

FIG. 1. Response of *Skeletonema costatum* to various concentrations of effluent and in filtered seawater controls (C). A, Kraft; B, newsprint; C, blend; D, single-pass; E, diethylether insoluble; F, diethylether soluble.

Table 3. Doubling times (hours) (*) for exponential phase of growth and chlorophyll *a* (micrograms per liter) (**) at termination of experiment in axenic culture experiments 1–14, at various concentrations of pulpmill effluent and in filtered seawater controls (C).

% effluent concn	*Skeletonema costatum*						*Dunaliella tertiolecta*				*Amphidinium carteri*			
	1	2	3	4	5	6	7	8	9	10	11	12	13	14
C1	16.3* 116.7**	16.0 205.9	22.5 112.1	16.6 78.4	20.3 55.1	11.4 38.4	25.2 844.3	28.9 989.5	26.1 905.1	26.5 994.5	24.0 342.9	24.0 218.4	34.2 207.8	33.0 322.3
C2	17.7 112.4	17.0 132.1	20.7 224.1	24.8 103.5	18.0 37.5	10.8 37.0	25.7 627.3	29.9 782.1	26.5 650.6	27.4 521.4	41.3 221.8	26.2 244.5	32.4 174.7	38.8 220.7
C3	15.7 70.2	17.0 169.9	20.9 134.7	19.6 97.7	19.0 30.2	10.8 23.9	24.9 361.2	28.9 342.4	27.3 355.2	27.9 449.7	43.4 238.5	31.7 237.0	33.0 227.7	20.7 188.7
C4	18.5 68.7	18.0 137.1	20.0 129.2	19.6 55.7	22.0 20.6	10.8 14.4	32.6 153.0	31.4 244.1	17.3 223.8	38.8 230.6	41.5 198.0	27.6 211.3	27.6 218.8	18.0 198.6
C5	— 58.0	— 89.2	— 97.7	— 66.4	— 26.3	— 20.7	— —	— —	— —	— —	— —	— —	— —	— —
10	18.0 201.0	19.2 210.7	19.7 185.0	20.4 193.3	19.2 49.2	10.3 23.9	— —	— —	— —	— —	37.1 384.7	25.2 373.6	28.9 442.7	45.1 304.5
20	22.8 103.8	25.9 131.1	20.9 194.8	16.7 202.6	18.4 31.9	10.1 27.6	— —	— —	— —	— —	35.0 305.1	24.0 309.3	38.5 365.0	39.8 273.3
30	19.5 28.7	18.0 107.9	— 10.0	17.0 153.7	32.3 0.0	22.2 32.4	24.8 994.8	28.9 1024.0	28.9 1032.0	28.9 1045.6	51.3 214.6	26.6 286.9	41.3 352.8	36.6 288.3
40	— 0.0	— 16.1	— 2.5	19.0 157.0	36.3 11.4	30.4 31.9	— —	— —	— —	— —	52.6 156.6	27.6 251.3	37.1 278.6	32.3 232.2
50	— —	— —	— —	— —	— —	— —	28.1 790.1	28.9 700.7	28.9 695.9	29.9 782.6	— —	— —	— —	— —
70	— —	— —	— —	— —	— —	— —	40.8 361.9	27.8 440.1	34.1 364.4	31.0 337.0	— —	— —	— —	— —
90	— —	— —	— —	— —	— —	— —	48.0 158.0	35.8 97.3	48.0 241.6	44.6 142.3	— —	— —	— —	— —

3A). Other effluents showed little effect on growth (Fig. 3 B–D). Doubling times increased with increasing effluent concentrations and chlorophyll *a* values decreased with increasing effluent concentrations (Table 3).

An experiment was conducted to determine if the effluent had lost toxicity on long-term storage (90 days). *Skeletonema costatum* was bioassayed in 40%, 90-day-old kraft and newsprint effluents, a concentration inhibitory in previous experiments. Results were identical to those of experiments 1 and 2 (Fig. 1A and 1B), suggesting no change in inhibitory capabilities after 90 days cold storage.

The manner in which effluents affected population growth was separated for discussion into lag, exponential, and stationary phase effects.

LAG

Prolonged lag phases were interpreted to mean that time was required for physiological adaptation prior to commencement of exponential growth. *Skeletonema costatum* and *A. carteri* generally required from several days to as long as 3 wk to adapt to higher concentrations of kraft effluent (Fig. 1A and 3A). In the other effluents, these species required little or no preadaptation. *Dunaliella tertiolecta* required no preadaptation to any effluent tested, even at highest concentrations.

EXPONENTIAL

An increase in the doubling time of a population from that of an appropriate control can be interpreted as growth inhibition. Kraft effluent affected the exponential growth rate of all species, increasing population doubling time with increasing effluent concentrations (Table 3). Single-pass effluent had the least effect on exponential growth of all species, while other effluents produced intermediate effects.

STATIONARY

The density of cells at the stationary phase reflects the degree to which the effluent has affected the exponential growth rate and/or contributed nutrients to sustain the exponential phase. Single-pass effluent produced the greatest density and yield (chlorophyll *a*), and kraft effluent the least. Remaining effluents gave intermediate effects (Table 3). In most experiments, increasing effluent concentrations decreased the density and yield of cells. Diethylether soluble fraction was the only exception to the above pattern showing identical yields of *S. costatum* at all concentrations tested.

Discussion

Kraft effluent had the greatest effect on growth of species tested, followed with diminishing effect by newsprint, blend, and single-pass effluents. Passage of blended kraft and newsprint effluent through an Amberlite XAD-8 resin column removed almost all inhibitory substances, since no effects on growth were seen in tests with this effluent, even at the highest concentrations. The diethylether soluble fraction of the methanol

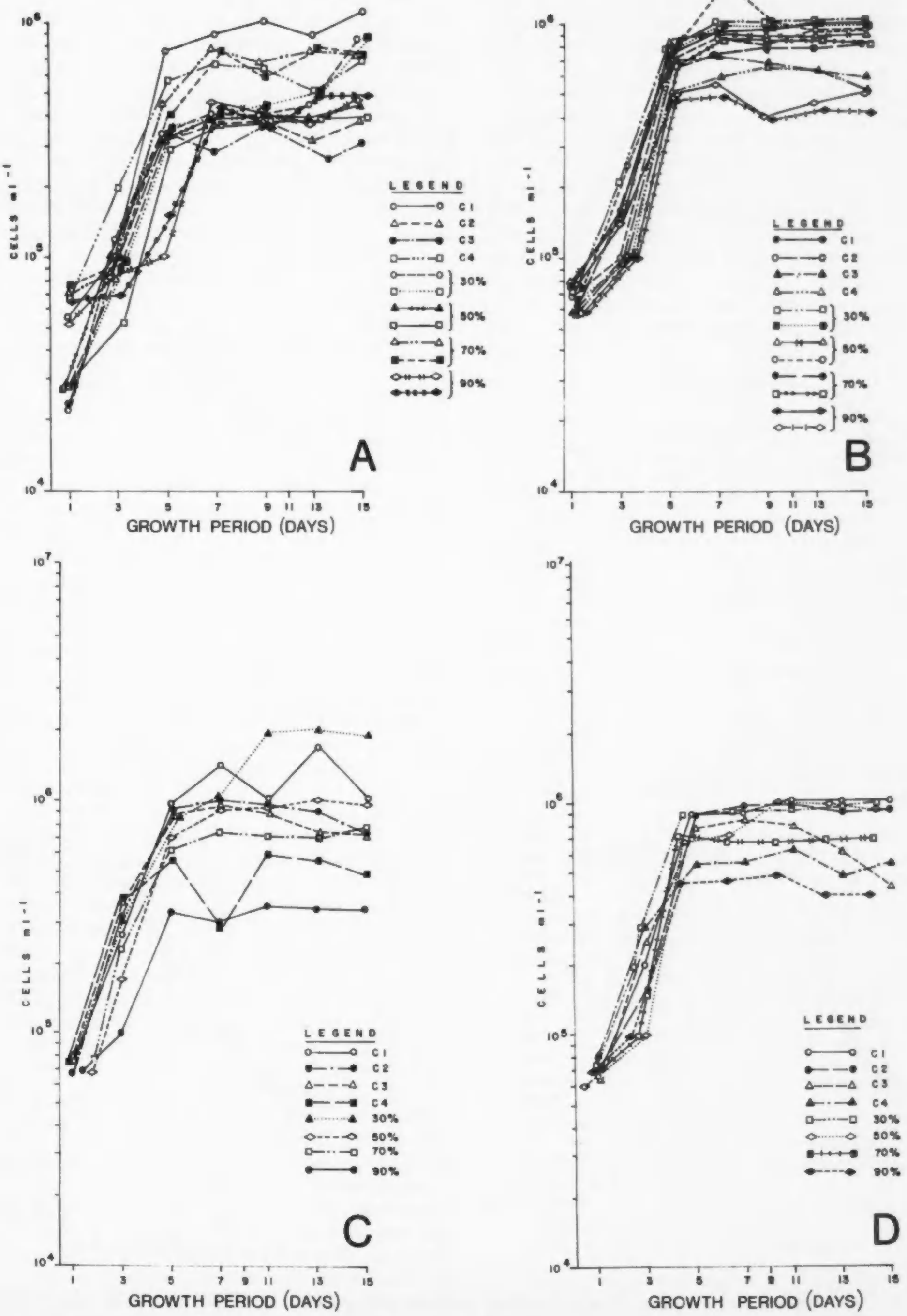

FIG. 2. Response of *Dunaliella tertiolecta* to various concentrations of effluent and in filtered seawater controls (C). A, Kraft; B, newsprint; C, blend; D, single-pass.

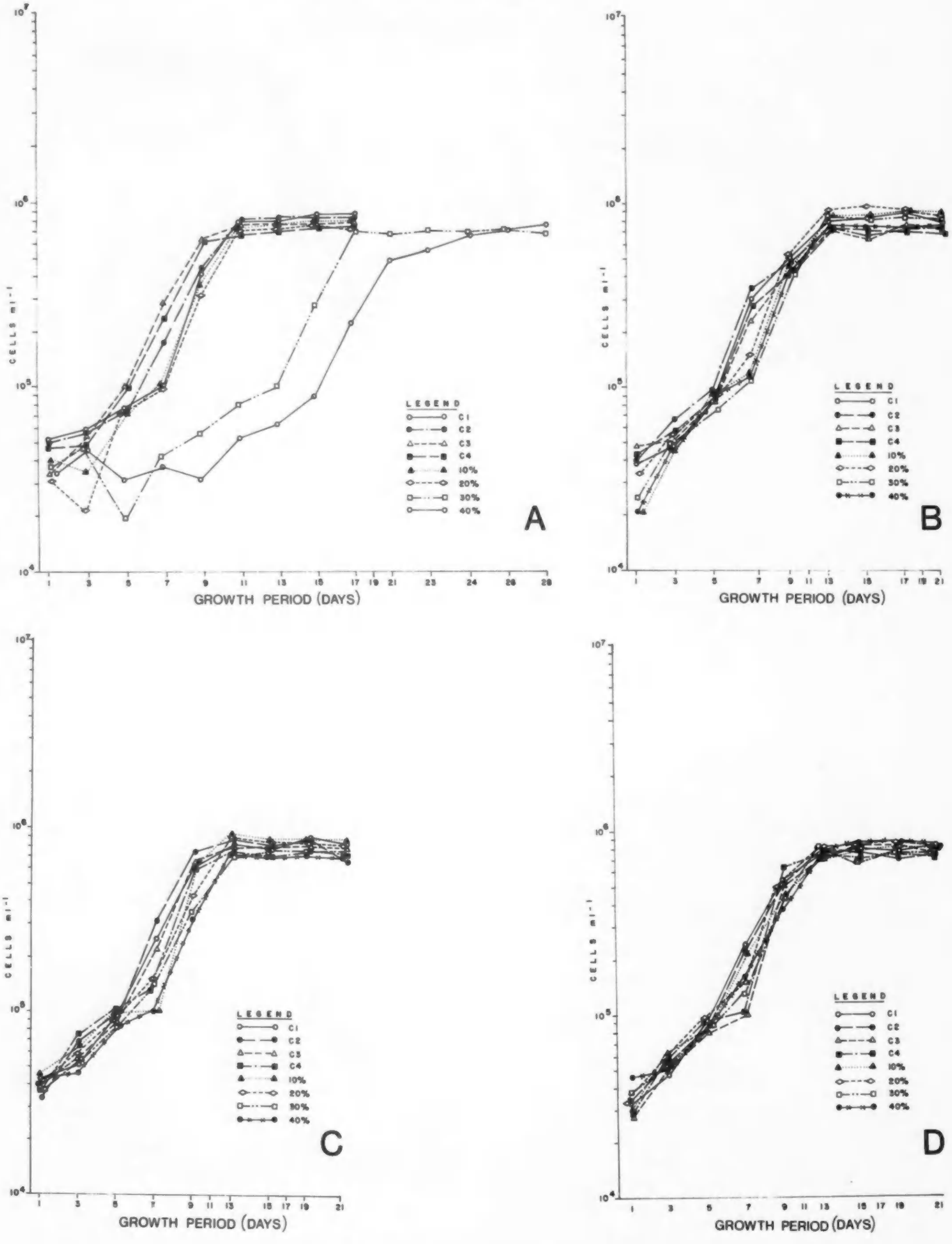

FIG. 3. Response of *Amphidinium carteri* to various concentrations of effluent and in filtered seawater controls (C). A, Kraft; B, newsprint; C, blend, D, single pass.

strippings from the column had little effect on growth, suggesting that wood extractives were not involved in the inhibition of growth of *S. costatum*. However, the diethylether insoluble fraction, containing lignin degradation products, affected growth in a manner similar to kraft effluent, except that no preadaptation period was required. It appears, on the basis of these experiments, that high molecular weight lignin derivatives are responsible for the growth-inhibiting effects seen in cultures of *S. costatum*, *A. carteri*, and to a lesser extent in *D. tertiolecta*.

Newsprint effluent had little effect on growth of the three species, except at the highest concentrations (40%), while the blend of the kraft with newsprint effluent gave intermediate effects, except in only one instance. That exception was *S. costatum* where the blend caused its most marked effect, lysing cells at 30 and 40% concentration (Fig. 1C). This suggests some type of negative synergistic effect when the two effluents are combined. Interestingly enough, Crofton pulpmill discharges both effluents together after mixing, i.e. as blend, and *S. costatum* is the dominant spring diatom in coastal waters!

Moore and Love (1976) in a short-term (4-h) bottle test demonstrated some growth inhibition of natural freshwater phytoplankton by the resin dehydroabietic acid. There were few resin acids present in our Crofton effluents (I. H. Rogers personal communication), and what were present would have appeared in the diethylether soluble fraction, which when tested with *S. costatum* produced no growth-inhibiting effects, even at 40% concentration.

Subject to the proviso that results of this study may be ecologically extrapolated only to eutrophic marine waters receiving kraft or newsprint pulpmill effluent without drastic pH change, our results suggest that growth of most coastal marine phytoplankton will not be seriously affected by effluent in the zone of influence, except when concentrations exceed 30–40%. If contact period between phyplankton cells and effluent is prolonged (days), adaptation to concentrations as high as 30–40% is possible for some species, but not all. Those species incapable of adaptation will either remain quiescent until light conditions improve or will be lost from the euphotic zone by sinking. When phytoplankton encounter high effluent concentrations (> 50%) cell lysis (death) will likely occur in some species, due either to substances in the effluent or to pH shock, or to a combination of both factors. If effluent concentrations encountered by phytoplankton after rapid incursion (hours) into and out of the zone of influence is below 30–40%, photosynthesis will again attain normal levels after sufficient time to readapt to new light conditions (Stockner et al. 1975; Parker and Sibert 1976). Eutrophication (growth enhancement) by nutrients (C, N, P) in effluent will occur in regions affected by low effluent concentrations where good light conditions prevail.

There are certain exceptions where these generalizations do not pertain. The first is in regions affected by effluents from sulfite process pulp mills, where evidence suggests growth inhibition (phytotoxicity) at low concentrations (< 10%) (Seppovaara and Numminen 1974; D. E. Goyette personal communication). The second is in situations where substances not normally discharged are accidently released in high concentrations (black liquor, lime sludge). In such cases, the entire biocenosis within the zone of influence will be seriously affected.

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AMERICAN PUBLIC HEALTH ASSOCIATION, AMERICAN WATER WORKS ASSOCIATION, AND WATER POLLUTION CONTROL FEDERATION. 1971. Standard methods for the examination of water and wastewater. 13th ed. New York, N.Y. 769 p.

FISHERIES ENVIRONMENTAL PROTECTION SERVICE. Laboratory manual. Dep. Environ., Fish. Mar. Serv., Pac. Reg. 261 p.

MOORE, J. E., AND R. J. LOVE. 1976. Effect of a pulp and paper mill effluent on the productivity of periphyton and phytoplankton. J. Fish. Res. Board Can. (In press)

PARKER, R. R., AND J. SIBERT. 1972. Effect of pulp mill effluent on dissolved oxygen in a stratified estuary — I. Empirical observations. Water Res. 7: 503–514.

1976. Responses of phytoplankton to renewed solar radiation in a stratified inlet. Water Res. 10: 123–128.

SEPPOVAARA, O., AND S. NUMMINEN. 1974. The effect of municipal and forest industry effluents upon algae production and the primary production ability of plankton. Papp. Tra 56: 523–530.

STOCKNER, J. G., D. D. CLIFF, AND K. MUNRO. 1975. The effects of pulpmill effluent on phytoplankton production in coastal waters of British Columbia. Fish. Mar. Serv. Dev. Tech. Rep. 578: 99 p.

STRICKLAND, J. D. H., AND T. R. PARSONS. 1968. A practical handbook of seawater analysis. Bull. Fish. Res. Board Can. 167: 311 p.

TULLY, J. P. 1949. Oceanography and prediction of pulpmill pollution in Alberni Inlet. Bull. Fish. Res. Board Can. 83: 169 p.

WALDICHUK, M. 1954. Effect of pulpmill waste in Alberni harbour. Fish. Res. Board Can. Prog. Rep. 101: 23–26.

Behavior and Growth of Juvenile Atlantic Salmon (*Salmo salar*) and Three Competitors at Two Stream Velocities

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SYMONS, P. E. K. 1976. Behavior and growth of juvenile Atlantic salmon (*Salmo salar*) and three competitors at two stream velocities. J. Fish. Res. Board Can. 33: 2766–2773.

In a laboratory stream juvenile Atlantic salmon (*Salmo salar*), common shiners (*Notropis cornutus*), blacknose dace (*Rhinichthys atratulus*), and white suckers (*Catostomus commersoni*) apparently preferred habitats that simulated their natural ones. Only salmon and one sexually mature male shiner defended territories. Dace were gregarious and nomadic, shiners tended to school in open water, and suckers were asocial bottom browsers.

No competitive advantage or disadvantage was apparent between species in capturing drift food and growing at fast and slow stream velocities. However, salmon, because of their aggressive territorial behavior, successfully competed for and reduced presence of other species in their preferred boulder habitat.

SYMONS, P. E. K. 1976. Behavior and growth of juvenile Atlantic salmon (*Salmo salar*) and three competitors at two stream velocities. J. Fish. Res. Board Can. 33: 2766–2773.

Dans un cours d'eau de laboratoire, les saumoneaux atlantiques (*Salmo salar*), les ménés à nageoires rouges (*Notropis cornutus*), les naseux noirs (*Rhinichthys atratulus*) et les meuniers noirs (*Catostomus commersoni*) semblent préférer des habitats qui ressemblent à leurs habitats naturels. Seulement les saumoneaux et un mené à nageoires rouges mâle sexuellement mûr défendent des territoires. Les naseux noirs sont grégaires et nomades, les ménés tendent à former des bancs en pleine eau, et les meuniers sont des brouteurs benthiques asociaux.

Il ne semble pas y avoir d'avantages ou de désavantages compétitifs entre espèces dans la capture d'une nourriture en dérive et dans la croissance, à de grandes ou de faibles vitesses de courant. Cependant, les saumons, à cause de leur comportement territorial agressif, défendent avec succès l'habitat de galets qu'ils préfèrent et en éloignent les autres espèces.

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ATLANTIC salmon (*Salmo salar*) juveniles frequently occur in the same streams as blacknose dace (*Rhinichthys atratulus*), common shiners (*Notropis cornutus*), creek chub (*Semotilus atromaculatus*), and white suckers (*Catostomus commersoni*). All species may occasionally be found in slow- (<30 cm/s) and fast-water habitats. Salmon, however, typically occur in rapidly flowing riffles, at least in summer (Gibson 1966; Elson 1967; Keenleyside 1962; Symons and Heland unpublished data) and they have a high metabolic rate compared with some other stream fishes (Clausen 1936; Lindroth 1942; Power 1959; Winberg 1960). Blacknose dace are common minnows of fast-flowing streams (Trautman 1957; Scott and Crossman 1973) though they also commonly occur along brush-covered banks in still waters (Symons and Harding 1974). Common shiners usually inhabit large open pools or lakes (Scott and Crossman 1973) where they frequently school, but a few may be found in streams where banks of grass and cobble offer shelter from the current (Symons and Harding 1974). Creek chub occur mostly in slow water where there is overhanging cover (Symons and Harding 1974). White suckers are classed by Scott and Crossman (1973) as fish of warmer, shallow lakes, or warm shallow bays and tributary rivers of larger lakes, but they also occur in pools, backwaters, and slow sections of some small streams.

The experiment described here is designed to test whether juvenile Atlantic salmon occur in fast water by choice or are forced there by competitors, and whether salmon have a competitive advantage in fast water compared with other species, competitive ability being measured by growth.

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Materials and Methods

The large artificial stream (Fig. 1) 70 cm deep consisting of two 15-m long parallel channels, one 92 cm wide, the other 119 cm, had an area of

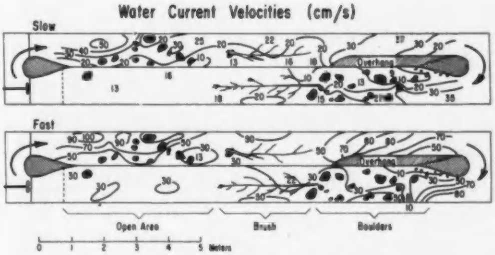


FIG. 1. Current velocities (centimeters per second) and habitats in the artificial stream.

26.6 m^2. A variety of bottom material and "cover" was used to construct four types of habitat: "boulder," "brush cover," "open," and "overhang." Two areas with boulders (25–40 cm diam, Fig. 2A and G) were located at the upstream ends of each channel (Fig. 1). Two areas of brush cover, each with a small dead tree fastened to the bottom, occupied the center section of each channel. The open area with pebble and gravel bottom was located at the downstream end of the wider channel. The overhang or cave at the foot of the narrow channel was constructed of plywood. It enclosed a portion of the side of the channel from above the water surface to the floor except at the downstream end where there was a space through which fish could move to the still water inside. Fish were only visible when outside the overhang or in the entrance space.

Current was created by a variable speed propeller. Two speeds were selected, one to provide currents ranging from 10 to 50 cm/s (average about 20 cm/s) and the other giving velocities of 10–100 cm/s (average about 40 cm/s). Current velocities were measured with an Ott current meter 5–15 cm above the bottom at three locations across the width of the stream at approximately 90-cm intervals. From these measurements, lines of equal current were drawn on a map of the stream (Fig. 1).

Juvenile Atlantic salmon were obtained from stocks at the Biological Station, St. Andrews, New Brunswick. Four species of "coarse fish" (white suckers, common shiners, blacknose dace, and creek chub) were collected as required by electrofishing from the Waweig River about 25 km from St. Andrews.

The stream was stocked with salmon alone, with salmon and coarse fish (all species), or with coarse fish (all species) but no salmon, and run at one of the two current velocities for 3 wk. At the end of 3 wk, the stream was drained and the fish collected. Fish were measured (total length, tail relaxed) and weighed before and after each run. Each fish was marked by hot or cold branding so that individual growth could be followed. Brands on salmon were distinguishable while the fish were in the stream, but on coarse fish the brands were usually only visible after fish were anesthetized with tertiary amyl-alcohol. The same individuals were used, except for replacement of mortalities, in the complete set of six runs (three species combinations × two speeds) allowing pairing of individual data from fast and slow waters. Runs were replicated with new fish, the total of six runs being repeated once, but the four runs with salmon alone and salmon plus coarse fish were replicated a third time. The order of fast and slow runs was reversed with each repetition except with salmon alone, where slow runs always preceded fast runs to reduce mortalities caused by inexperienced fish being swept through the propellor or against the screen guarding it. Runs were performed at all seasons over a period of 1½ yr. Efforts were made to minimize seasonal growth variations by maintaining a photoperiod of 12 h light and temperatures of 12–15 C through the winter, but in summer light from windows in the building extended the photoperiod, and temperatures in July and August rose to 17 C.

When salmon were used in the stream, 10 individuals were introduced (average individual weights 4.42–12.28 g). Coarse fish per run numbered from 5 to 11 dace (average weight 1.8–2.9 g), 0 to 6 chub (3.3–9.6 g), 5 to 8 suckers (6.0–13.8 g), and 3 to 8 shiners (average 1.9–7.9 g). Population densities by weight of shiners and suckers in the artificial stream approximated those estimated in the Waweig River (Symons and Harding 1974). Dace were stocked at approximately half the density estimated for the Waweig, and chub at half or less.

A variety of food particle sizes was provided by two types of food, brine shrimp (*Artemia* sp.) and mealworms (*Tenebrio* sp.). Packages of commercially frozen brine shrimp were thawed and drained for 5–10 min in a fine-mesh net, then divided into portions of about 4 g and refrozen until required. Each day one 4-g portion of brine shrimp and 1.0 g of mealworm were fed to the fish. The shrimp were either prethawed or the frozen block was withheld in a corner of the stream so that brine shrimp were released and swept downstream individually. The live mealworms were distributed among four "feeders" consisting of a pair of plastic containers, one inside the other, with holes in the bottoms through which the mealworms tumbled into the stream during the day. Usually, at least 30 mealworms were required to make up the daily ration. Attempts were made to provide a variety of sizes at each feeding, but mealworms nonetheless were usually too large to be eaten by the smaller fish. A trial run indicated that this quantity and variety of food were sufficient at the slow speed to maintain the fish in good condition and allow a slight increase in average weight. The ration was not altered to match the biomass of salmon or course fish when alone or in combination since the experiment was designed primarily to test for differences in weight gain or loss at the two current velocities rather than to test between absolute growth rates.

The location of all visible fish was recorded twice daily on weekdays, once on weekends, in all three pairs of fast and slow runs with salmon and coarse fish together, but, to reduce labor, in only one of two pairs of fast and slow runs with coarse fish alone, and in two of the three pairs of runs with salmon

TABLE 1. Distance (centimeters) ± SE between extremes of excursions up- and downstream during 10-min observations (number of fish or number of distinctive size-classes of fish when not individually identifiable, in parentheses) using the same fish at fast and slow stream velocities. *$P < 0.05$, difference significant using Student's t-test; $0.05 <$ ** $P < 0.1$.

	Salmon		Shiners		Dace		Suckers	
	Slow	Fast	Slow	Fast	Slow	Fast	Slow	Fast
Salmon alone	240±33 ** (18)	179±27 (18)						
Coarse fish alone			341±205 (4) **	42±42 (2)	392±73 (3) **	204±41 (6)	255±17 (3)	226±59 (6)
Salmon and coarse fish	290±21 * (25)	151±22 (21)	139±40 (9)	40±3 (3)	189±65 (7)	177±34 (12)	169±49 (7)	119±61 (3)

alone. The first daily observation, on weekends the only one, was made immediately after feeding at 0800–1000 and the second between 1600 and 1630. On the 2 days preceding termination of the run in those runs where daily observations had been made, the locations of fish were recorded hourly between 0800 and 1600. In addition, on these 2 days observations were recorded of the movements and behavioral interactions of most visible individuals for 10 min each. Significant differences ($P < 0.05$) were calculated using t-tests.

Results

GENERAL BEHAVIOR OF SALMON AND COARSE FISH

Salmon were territorial as described by Kalleberg (1958) and Keenleyside and Yamamoto (1958). They defended territories against other salmon and occasionally individuals of other species by displaying, nipping, and chasing. Interactions of salmon with dace, shiners, and suckers were occasionally observed. In these encounters, salmon were always the aggressors and frequently the result was the departure of the other species. Possibly as a result of this aggression, movement of coarse fish was consistently lower in runs with salmon, but not significantly so (Table 1). Coarse fish appeared to keep a meter or so distant from salmon where they were usually ignored.

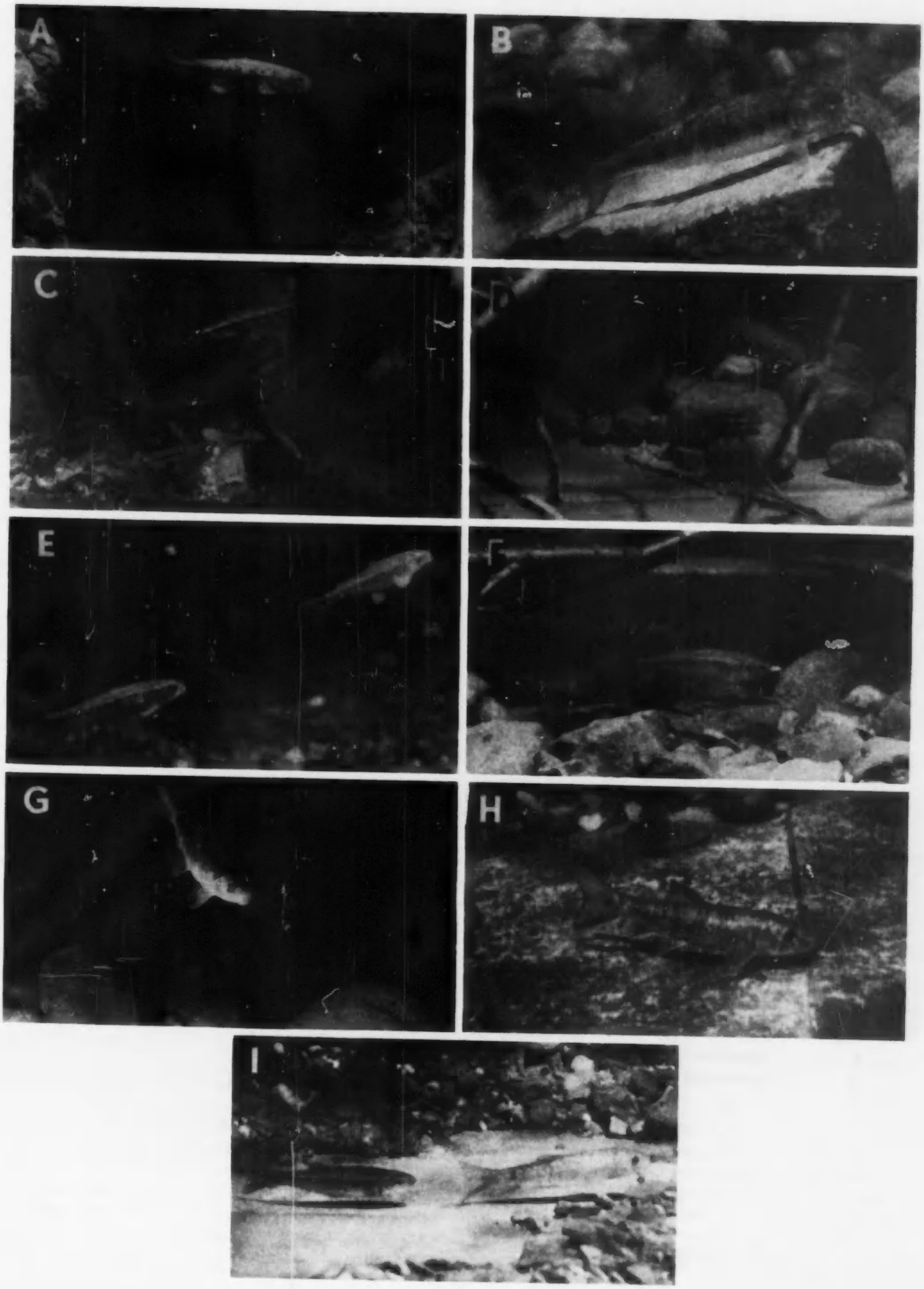
At fast water velocities with coarse fish present, salmon made significantly shorter excursions up- and downstream than they did at slow water velocities. Reduction of movement at fast velocities was also consistent among all species, although with the exception noted, differences were not significant (Table 1).

To provide a quantitative evaluation of habitat preferences of the different species, the number of sightings of individuals of each species from each daily or twice daily observation was totaled for the boulder, brush-cover, and open habitats and expressed as a percentage of total potential sightings (= numbers of individuals of that species present in the stream at the time of observation times the number of daily observations in the run).

Under all conditions salmon were sighted most frequently (23–38%) in the boulder habitats (Fig. 3). Their second choice appeared to be the open habitat. Salmon spent most of their time holding position in their territories either by constant swimming (Fig. 2A) where the current was slow (20–30 cm/s) or by using their pectoral fins as planes to maintain contact on top of a boulder in swifter water (Fig. 2B) as described by Stuart (1953) and Kalleberg (1958). Sometimes salmon would hold in the relatively still water immediately in front of a boulder (Fig. 2A), but only occasionally did small salmon entrain behind objects, as described by Sutterlin and Waddy (1975) for brook trout (*Salvelinus fontinalis*), and large ones were never observed to do so. Salmon fed on drifting mealworms and brine shrimp either at the surface or in midwater.

Dace were nonterritorial, nomadic, and gregarious, frequently moving in pairs (Fig. 2C) or

FIG. 2. Fishes in various habitats of the artificial stream in slow (left column) and fast (right column and bottom) runs. Atlantic salmon (*Salmo salar*): A, in boulder habitat and B, holding on rock with pectoral fins; blacknose dace (*Rhinichthys atratulus*): C, two in company off bottom and D, near bottom in brush cover; common shiner (*Notropis cornutus*): E, aggregating off bottom in open habitat and F, eating mealworm in brush cover; white sucker (*Catostomus commersoni*): G, catching drift food in boulder habitat and H, feeding normally on bottom; and white sucker and creek chub (*Semotilus atromaculatus*): I, holding in fast current open habitat.


A
B
C
D
E
F
G
H
I

small groups for short periods of time. They shifted their "home-station" every few days, a "home-station" being an undefended area within which a fish spent the majority of time each day. Though dace were the smallest species, they ranged up- and downstream as far as other species except salmon ($P < 0.05$) when together with them at slow velocities (Table 1).

In the absence of salmon, dace were sighted most frequently (30–36%) in the boulder habitat (Fig. 3). They spent most of their time patrolling over, around, and under rocks, visually inspecting them, perhaps for food, though only algae grew on the rocks in the artificial stream. Dace appeared able to maneuver readily among boulders in fast currents. Occasional forays were made to midwater and surface. Dace fed on brine shrimp, catching them as drift and perhaps also picking them off the bottom from between the rocks though this could not be confirmed by observation. Only the smallest mealworms could be eaten by dace.

At the slow current speed, shiners could be seen in midwater or near the surface especially in the sections with brush cover (Fig. 3). One large male defended a territory for about 2 wk, but the others were not aggressive. Smaller individuals formed aggregations (in the terminology of Breder [1959]) (Fig. 2E). Occasional sightings of them at the opening to the overhang at fast velocities suggested they remained out of sight in the still water within. Shiners fed voraciously on brine shrimp. The larger shiners also ate a few mealworms (Fig. 2F).

Suckers were nonterritorial, generally asocial, slow-swimming, bottom browsers. They spent 80–90% of their time scraping at rocks with their mouths (Fig. 2H). The other 10–20% was spent quietly resting on the bottom. Suckers occasionally left the bottom to try to catch drifting brine shrimp (Fig. 2G), but their main source of food was probably mealworms which, when missed by other species, sank to the bottom and fell between the rocks. In slow runs suckers were common in the brush-cover habitat. In fast runs suckers lay motionless and all but invisible between the rocks or moved out of sight into the overhang. They appeared to have more trouble maintaining equilibrium in fast or turbulent water than the other species.

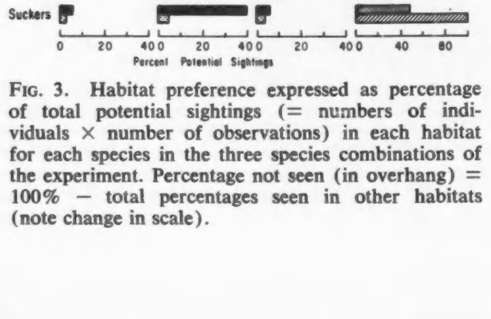


FIG. 3. Habitat preference expressed as percentage of total potential sightings (= numbers of individuals × number of observations) in each habitat for each species in the three species combinations of the experiment. Percentage not seen (in overhang) = 100% − total percentages seen in other habitats (note change in scale).

Chub were the most secretive of the five species, apparently spending most of their time in the overhang where they were only occasionally visible at the entrance. Because only a few chub were used and these were seldom seen, nothing further will be said of their behavior.

HABITAT PREFERENCE INFLUENCED BY OTHER SPECIES

In the presence of salmon the proportion of potential sightings when coarse fish were seen amongst boulders or in the open, the favored habitats of salmon decreased (Fig. 3). Dace, shiners, and suckers moved to brush cover, the habitat where salmon were least frequently seen, or moved out of sight presumably into the overhang. Salmon appeared to influence the distribution of coarse fish between habitats, but coarse fish had little influence on the presence or absence of salmon in a particular habitat (Fig. 3).

LOCATION OF FISH WITH RESPECT TO CURRENT VELOCITY

The majority of sightings of each species except salmon in slow runs was in the modal velocity for the stream, which was 10–19 cm/s in slow runs and 30–49 cm/s in fast runs. However, most of the sightings of salmon in slow runs (52–54%) occurred in currents exceeding 19 cm/s (Table 2). Suckers in slow runs were sighted more frequently than other species in the slowest currents.

GROWTH

Growth, expressed as change in weight per unit weight of individual per 100 days (instantaneous growth rates could not be used because many fish lost weight), was less for all species in fast runs than in slow runs. Comparing runs with and without coarse fish or salmon separately, this decrease was always significant except for salmon in the absence of coarse fish (Table 3). Suckers actually lost weight in fast currents.

TABLE 2. Location of fish with respect to current velocity expressed as percent of sightings for each species when salmon were alone or with coarse fish, coarse fish were alone or with salmon.

	% sightings slow stream velocities (*cm/s*)					% sightings fast stream velocities (*cm/s*)				
	< 10	10–19	> 19	Total sightings	No. fish	< 29	30–49	> 49	Total sightings	No. fish
Salmon										
alone	2	44	54	649	18	20	61	19	583	18
with coarse fish	1	47	52	757	28	20	66	14	740	26
Dace										
coarse fish alone	5	52	43	164	6	16	77	7	194	6
with salmon	0	73	27	245	17	26	68	6	419	12
Shiners										
coarse fish alone	2	52	46	147	4	13	55	32	72	3
with salmon	1	80	19	331	15	39	56	5	168	9
Suckers										
coarse fish alone	8	65	26	260	8	18	80	2	119	6
with salmon	4	77	19	321	16	24	71	5	83	13

TABLE 3. Average growth (grams per gram of fish per 100 days) at fast and slow currents with and without potential competitors, number in sample is in parentheses. An asterisk between two averages indicates a significant difference ($P < 0.05$ using Student's *t*-test).

Salmon	Alone		With coarse fish[a]
fast	+1.03±0.17 (28)		+0.99±0.15 (21)
slow	+1.23±0.12 (29)		+1.23±0.17 (25)
Coarse fish	Without salmon		With salmon
Shiners			
fast	+0.16±0.15 (9)		+0.10±0.15 (10)
	*		*
slow	+0.75±0.21 (11)		+0.44±0.12 (14)
Dace			
fast	+0.12±0.08 (12)	*	−0.98±0.07 (21)
	*		*
slow	+0.40±0.06 (12)		+0.30±0.09 (15)
Suckers[b]			
fast	−0.01±0.00 (11)	*	−0.07±0.08 (13)
	*		*
slow	+0.37±0.34 (13)	*	+0.60±0.11 (16)

[a] The number of individual salmon that grew slower in fast current than in slow was not significant with a *t*-test but was sufficiently consistent to be significant ($P = 0.032$, Sign test, $n = 19$, $\bar{x} = 5$).

[b] In the case of suckers only, the decrease in growth in fast compared with slow currents was significantly greater in the presence of salmon than in their absence (Fig. 4).

Growth of salmon was little affected by the presence or absence of coarse fish (Table 3). In contrast, the presence of salmon in fast velocities slowed the growth of dace and suckers. Both species lost weight in fast runs with salmon present. In slow currents, however, suckers grew significantly faster when salmon were present. The reason for this reversal in expected pattern was not clear. Since the three runs with coarse fish and salmon fell by chance in spring and summer (March, June, and August), while the two runs without salmon occurred in winter (November and January), there was a seasonal difference. This, coupled perhaps with an ability of suckers to utilize algae, may have permitted them to grow independently of supplied food and potential competitors.

To compare the ability of each species to compete for food and to grow at the two stream velocities, the relative growth rates in fast and slow currents were more important than the absolute growth rates which differed inherently between species (Table 3) and perhaps even between individuals. To cancel the effect of inherent growth differences, therefore, the difference in growth of each individual present in fast and slow runs was calculated. These individual differences were then averaged and the standard error calculated for each species.

In the absence of salmon the growth of all three coarse fish was significantly depressed by fast stream velocities (Fig. 4). There was little difference between species. Depression in growth of salmon by fast water in the absence of coarse fish was not significant, but neither did salmon, when alone, perform better in fast currents than coarse fish without salmon (Fig. 4). In the face of competition from salmon, growth of all three coarse fish was again significantly depressed by fast water, but, except for suckers, the magnitude of this depression was not increased. Growth of suckers in the presence of salmon was depressed by fast currents significantly more than when salmon were absent, but at least a part of this difference was due to better growth of suckers in slow water with salmon than in slow water without (Table 3). This difference was the result of an uncontrolled, perhaps seasonal, effect as discussed above, and must therefore be interpreted cautiously.

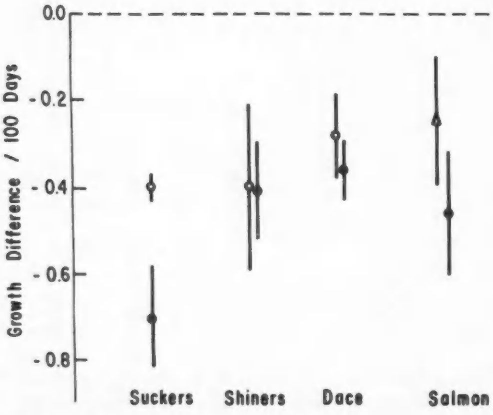


FIG. 4. Depression in growth rate by fast velocities determined by difference in growth in fast minus slow velocities. ○, coarse fish in the absence of salmon; △, salmon in the absence of coarse fish; ●, salmon and coarse fish together.

Discussion

The experiment demonstrated that, in this particular fish community, salmon could compete successfully for their preferred boulder habitat with coarse fish. This conclusion was based on the movement of dace and suckers out of boulder areas when salmon were present, coupled with observations of salmon chasing and nipping members of other species. The dace, shiners, and suckers, with the exception of one shiner, were neither aggressive nor territorial. The success of salmon in competing for space with the other species apparently stemmed from their aggressive territorial behavior.

Allen (1969) suggested that most stream fish were solitary and territorial, a behavior that aided in division of food. However, in the artificial stream only Atlantic salmon were regularly territorial. Territorial behavior may be a characteristic of salmonids rather than of stream fishes in general.

The habitats in which each species was most frequently seen corresponded in general with that described as the preferred habitat in the field. In the artificial stream salmon and dace were seen most frequently in the boulder habitat. Dace in the presence of salmon usually occurred in brush cover. Shiners appeared to avoid rapid currents by moving out of sight into the still water of the overhang. Because there were no large open spaces. shiners apparently had to utilize habitats which, in the field, they would only have visited rarely. Suckers in the artificial stream, as in natural streams, preferred areas with slow flow.

Ability of fish to swim rapidly is related to body form (Alexander 1967; Allen 1969; Arnold 1974; Webb 1975) and to size (Bainbridge 1960). Brett and Glass (1973) have shown in laboratory studies that the 60-min critical swimming speeds of sockeye salmon (*Oncorhynchus nerka*) in the 7–12 cm size range are about 5 body lengths/s (12–15 C). Everest and Chapman (1972) found a correlation of +0.92 between lengths of juvenile chinook salmon (*O. tshawytscha*) and the water velocity at the focal points of their territories. On the basis of size alone, suckers in the present experiments should have occupied the same velocities as Atlantic salmon; that they did not, reinforces the known preference of suckers for slow water.

Although the experiment demonstrated clearly the preference of salmon for boulder habitats and also their ability to compete successfully for this space, it did not demonstrate that salmon could compete for food and grow better in fast water compared with species normally found in slower water. This failure may have arisen through details of experimental design. The salmon were of hatchery stock raised in slow-flowing water and they may not have performed as well in fast waters as wild fish would have. Secondly, size differences among fish species may have reduced competition for food as each was able to specialize on a food particle size most suited to it. However, feeding ability and energy consumption of different species may not be differentially affected by water velocity. In this event "fast-water species" may prefer fast water because it offers a refuge from predators.

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Alexander, R. M. 1967. Functional design in fishes. Hutchinson and Co. (Publishers) Ltd., London. 160 p.

Allen, K. R. 1969. Distinctive aspects of the ecology of stream fishes: a review. J. Fish. Res. Board Can. 26: 1429–1438.

Arnold, G. P. 1974. Rheotropism in fishes. Biol. Rev. 49: 515–576.

Bainbridge, R. 1960. Speed and stamina in three fishes. J. Exp. Biol. 37: 129–153.

Breder, C. M. 1959. Studies on social groupings in fishes. Bull. Am. Mus. Nat. Hist. 117: 393–482.

Brett, J. R., and N. R. Glass. 1973. Metabolic rates and critical swimming speeds of sockeye salmon (*Oncorhynchus nerka*) in relation to size and temperature. J. Fish. Res. Board Can. 30: 379–387.

Clausen, R. G. 1936. Oxygen consumption in freshwater fishes. Ecology 17: 216–226.

Elson, P. F. 1967. Effects on wild young salmon of spraying DDT over New Brunswick forests. J. Fish. Res. Board Can. 24: 731–767.

Everest, F. H., and D. W. Chapman. 1972. Habitat selection and spatial interaction by juvenile chinook salmon and steelhead trout in two Idaho streams. J. Fish. Res. Board Can. 29: 91–100.

Gibson, R. J. 1966. Some factors influencing the distribution of brook trout and young Atlantic salmon. J. Fish. Res. Board Can. 23: 1977–1980.

Kalleberg, H. 1958. Observations in a stream tank of territoriality and competition in juvenile salmon and trout (*Salmo salar* L. and *S. trutta* L.). Inst. Freshwater Res. Drottningholm Rep. 39: 55–98.

Keenleyside, M. H. A. 1962. Skin-diving observations of Atlantic salmon and brook trout in the Miramichi River, New Brunswick. J. Fish. Res. Board Can. 19: 625–634.

Keenleyside, M. H. A., and F. T. Yamamoto. 1958. Territorial behavior of juvenile Atlantic salmon (*Salmo salar* L.). Behaviour 19: 139–169.

Lindroth, A. 1942. Sauerstofferbrauch der Fische. II. Verschiedene Entwicklungsund Altersstadien vom Lachs und Hecht. Z. Vgl. Physiol. 29: 583–594.

Power, G. 1959. Field measurements of the basal oxygen consumption of Atlantic salmon parr and smolts. Arctic 12: 194–202.

Scott, W. B., and E. J. Crossman. 1973. Freshwater fishes of Canada. Bull. Fish. Res. Board Can. 184: 966 p.

Stuart, T. A. 1953. Spawning migration, reproduction, and young stages of loch trout (*Salmo trutta* L.). Freshwater Salmon Fish. Res. Scotland 5: 1–39.

Sutterlin, A. M., and S. Waddy. 1975. Possible role of the posterior lateral line in obstacle entrainment by brook trout (*Salvelinus fontinalis*). J. Fish. Res. Board Can. 32: 2441–2446.

Symons, P. E. K., and G. D. Harding. 1974. Biomass changes of stream fishes after forest spraying with the insecticide fenitrothion. Fish. Res. Board Can. Tech. Rep. 432: 47 p.

Trautman, M. B. 1957. The fishes of Ohio with illustrated keys. Ohio State University Press, Columbia, Ohio. 683 p.

Webb, P. W. 1975. Hydrodynamics and energetics of fish propulsion. Bull. Fish. Res. Board Can. 190: 158 p.

Winberg, G. G. 1960. Rate of metabolism and food requirements of fishes. Nauchn. Tr. Belorunk. Gos. Univ. Im. Vopr. Issled. Lenina, Minsk. 253 p. (Transl. from Russian by Fish. Mar. Serv. Transl. Ser. No. 194, 1960).

Uptake and Retention of Kuwait Crude Oil and Its Effects on Oxygen Uptake by the Soft-Shell Clam, *Mya arenaria* [1,2]

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FONG, W. C. 1976. Uptake and retention of Kuwait crude oil and its effects on oxygen uptake by the soft-shell clam, *Mya arenaria*. J. Fish. Res. Board Can. 33: 2774–2780.

Mya arenaria gills are capable of removing finely dispersed oil globules of 1–30 μm diam from sea water along with food particles. Clams exposed to seawater concentrations of 90–380 μg oil/liter for 10 days at 18 C accumulated oil in their tissues. Concentrations incorporated are directly related to the lipid content of tissues. Approximately 6 mg oil/g lipid was observed within the tissue of individual clams. Respiration rates of small clams (shell length 3.75–4.50 cm) at 18 C are significantly increased after 3 wk of exposure to sea water containing 220–370 μg oil/liter.

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Les branchies de *Mya arenaria* sont capables d'extraire de l'eau de mer, en même temps que les particules de nourriture, des globules de pétrole finement dispersés de 1–30 μm de diamètre. Les coques exposées à de l'eau de mer contenant 90–380 μg de pétrole/litre pendant 10 jours à 18 C accumulent du pétrole dans leurs tissus. Les concentrations qu'elles assimilent sont directement liées à la teneur en lipides des tissus. Nous avons observé dans le tissu de coques individuelles approximativement 6 mg de pétrole/g de lipides. Les taux respiratoires de petites coques (longueur de coquille de 3.75–4.50 cm) à 18 C sont significativement plus élevés après 3 semaines d'exposition à de l'eau de mer contenant 220–370 μg de pétrole/litre.

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THE accumulation of petroleum hydrocarbons in shellfish exposed to oil pollution has been well established (Cahnmann and Kuratsune 1957; Blumer et al. 1970; Erhardt 1972; Lee et al. 1972; Stegeman and Teal 1973). Two possible pathways of uptake have been suggested: a) consumption of contaminated food particles through feeding activity, and b) equilibration of tissues, particularly the gill surface, with dissolved hydrocarbons in sea water. The first suggestion is supported by the experimental results of Stainken (1975) who observed that the clams handled dyed oil globules as food and detritus particles. Evidence for the second suggestion was provided by Lee et al. (1972). They reported that hydrocarbons were taken up by gill tissue more rapidly than many others and hypothesized that the gill tissue of mussels has a layer that absorbs hydrocarbons and then passes them to other tissues. However, the relative importance of the two pathways has not been determined.

The rate and extent of accumulation of petroleum hydrocarbons have been found to be affected by the lipid content of the shellfish (Stegeman and Teal 1973). However, an early proposal by Blumer et al. (1970) has not been tested. Their proposal that incorporated oil would become part of the lipid pool of an organism has been one of the main questions examined in this work.

Considerable confusion exists concerning the effects of oil on marine life. In shellfish, apparently low concentrations of oil increase respiration rate, while high concentrations lowered oxygen uptake (Gilfillan 1975; Dunning and Major 1974). Little is known about the metabolic effects of oil on clams. The investigation reported here also deals with the acute and prolonged exposure effects of oil on oxygen utilization by *Mya arenaria.*

Materials and Methods

Mya arenaria were collected from the intertidal zone at Three Fathom Harbour, Nova Scotia, in late August 1974. The clams were acclimatized 1 wk in

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running sea water, at either 15 or 18 C, before testing. All experiments were completed within 30 days.

UPTAKE OF OIL PARTICLES

Oil contaminated sea water was prepared by adding one drop of Kuwait crude oil to a liter of filtered (0.45 μm) sea water and shaking 300 times in a 6 liter separatory funnel. After allowing to stand 1 h, during which the large globules rose to the surface and coalesced to form a film, the concentration of oil in colloidal suspension in the water (estimated by fluorescence spectroscopy, see below) was 7 mg/liter. Particle sizes, determined using a coulter counter, ranged from 1 to 32 μm in a log normal distribution. The water was added to an exposed gill at this point, and the response of the gill to oil globules was studied by fluorescence microscopy. Epi-illumination allowed observation of intact animals with one side of the shell and mantle removed.

The ability of clams to retain particles of the size typical of oil droplets was tested using the technique and apparatus described by Vahl (1972a, b; 1973).

DISTRIBUTION OF ASSIMILATED PETROLEUM HYDROCARBONS IN CLAM TISSUES AND CORRECTION WITH LIPID CONTENT

Redistilled ACS grade solvents were used in the determination of hydrocarbon concentrations in clam tissues. All of the apparatus and instruments were solvent washed.

Clams were allowed to bury themselves in the experimental tank (75 × 40 × 33 cm), supplied with unfiltered sea water at 500 ml/min at 18 C and stirred by aeration. Previous experience has shown the water to contain adequate natural food. Kuwait crude oil was added at a constant rate with an infusion pump. Water samples (100 ml) were periodically collected for hydrocarbon analysis, avoiding contamination from the surface film. These were extracted twice with 10 ml of methylene chloride in a 250 ml separatory funnel. The combined extracts were concentrated in a rotary evaporator, under reduced pressure at 30 C, and then redissolved in 10 ml of *n*-hexane. The oil concentrations were estimated by fluorescence spectroscopy (Keizer and Gordon 1973) and ranged from 90 to 380 μg/liter.

After 10 days exposure to oil contaminated sea water, four clams (shell length approximately 5.50 cm) were gathered and washed under cold tap water to remove sand particles. Intact clams were then dipped in a beaker of methylene chloride for a few seconds to prevent contamination from shell surfaces during dissection. Midgut and rectum, hepatopancreas, gonad, foot and adductor muscles, mantle, and gill were dissected and dipped in *n*-hexane for about 2 s to remove any possible surface oil contamination. After draining on filter paper, approximately 0.15 g of each tissue was homogenized for 60 s in 30 ml of *n*-hexane. The soluble fraction was obtained by filtering through solvent washed Whatman No. 1 filter paper which was then rinsed using 20 ml of *n*-hexane. The combined filtrate (about 50 ml) was reduced to about 0.5 ml in a rotary evaporator and redissolved in *n*-hexane at a ratio of 0.1 g of tissue (wet wt)/10 ml of *n*-hexane.

Synchronous excitation of emission spectra (Lloyd 1971) was obtained with a Perkin-Elmer (Model MPF-2A) Fluorescence Spectrophotometer (excitation: 200–427 nm; emission: 223–450 nm; slit widths: 6 nm) for the contaminated tissues, control tissues, Kuwait crude oil, and *n*-hexane blanks. Estimates of oil concentration in different tissues were obtained using the same analytical procedure as that for contaminated sea water. The controls were used to check the background fluorescence that was not from oil (Hargrave and Phillips 1975).

The concentration of lipid in various organs was determined in 20 clams having a shell length of about 5.5 cm using the modified method of Bligh and Dyer (1959) and Folch et al. (1957). Relative wet weight of the various tissues of a clam was estimated by dissecting 40 clams.

EFFECT OF KUWAIT CRUDE OIL ON THE RESPIRATION OF CLAMS

The variation in oxygen uptake was chosen as a convenient index of sublethal effect of oil on clams, as was previously done by Hargrave and Newcombe (1973) in snails. Two techniques were used to reduce variation in respiration measurement because the rate of oxygen uptake varies greatly according to whether the shell is opened or closed (van Dam 1935). One was to test short-term effects on pieces of isolated gill tissues. Preliminary experiments showed that the oxygen consumption of gill tissues was consistent for pieces of the same surface area. The gill of an adult clam (shell length about 6 cm) was cut into discs of equal area. One half of these were treated with either 0.7 or 7 mg/liter contaminated sea water (filtered, 0.45 μm); the other half (control) were placed in filtered sea water (0.45 μm) without oil. After 1 h both series were transferred into 30-ml glass stoppered flasks filled, by siphoning, with filtered sea water (0.45 μm). The flasks were kept in constant rotation and oxygen uptake was determined over a period of 3–4 h, at 10 C, with a radiometer Blood Gas Analyzer.

The other technique used to reduce variation in respiration measurements was to hold intact animals in moist air before returning them to water for oxygen uptake measurements. This artificially severe stress resulted in higher (Collip 1921) and more constant oxygen uptakes rates (unpublished data). It ensured that the clams exchanged gases with their maximum capacities after the build-up of oxygen debts and reduced variability caused by closing the valves. Clams were exposed to contaminated sea water in the same apparatus as described for oil retention experiments. Two experiments under different conditions were performed. In the first, 22 clams of various sizes were exposed to contaminated sea water with oil concentrations from 39 to 128 μg/liter for 30 days at 15 C. In the second experiment, 23 small clams (shell length 3.75–4.50 cm) were exposed to oil concentrations between 220 and 370

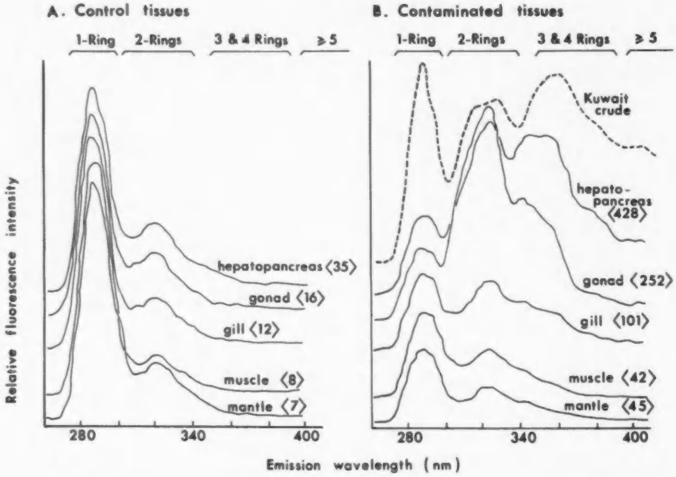

FIG. 1. Comparison of synchronous excitation of emission spectra of (A) control tissues and (B) those contaminated with Kuwait oil. Estimates of level of Kuwait crude oil equivalent in tissues are given in brackets (micrograms of oil per gram wet weight). The standard Kuwait crude oil displaying the spectrum has a concentration of 44 μg/10 ml *n*-hexane. The spectrum of contaminated-intestine extract is off the scale. The number of rings in the aromatic compounds producing the major peaks is identified by Gordon and Keizer (1974).

μg/liter for 21 days at 18 C. After treatment, clams were transferred to glass jars (900 ml) and kept for 18 h without sea water. A little water was splashed into each jar to keep the atmosphere moist. The jars were then filled with fresh uncontaminated sea water of reasonably constant particle concentration for oxygen uptake measurements. Clams were given 15 min to open their valves and release the air trapped inside their mantle cavities. Oxygen consumption by single clams was then measured for 4–6 h, between 0900 and 1500.

Results

The response of an exposed gill to oil globules as observed by fluorescence microscopy is similar to that described by Stainken (1975), who used dyed oil. Oil globules did not adhere to gill cilia or clog the gill. The clams handled oil globules as food particles. Furthermore, clams will retain particles typical of oil globules (1–32 μm); the maximum retention efficiency of particles by clams was about 50% for particles larger than 6 μm diam.

The spectra displayed by *n*-hexane extracts of contaminated and control tissues in comparison to Kuwait crude oil are shown in Fig. 1. The spectra of contaminated tissues resemble that of Kuwait crude oil, with a large increase in compounds with a high number of benzene rings when compared with control tissues. Kuwait crude oil equivalent in various tissues of contaminated clams decreases in the following order: intestine, hepatopancreas, gonad, gill, muscle, and mantle and each is at least 10 times those in corresponding tissue of control animals (Table 1). Blanks showed the contamination due to experimental procedure is negligible (<1 μg/sample).

The concentration of lipid and relative proportion by wet weight of various organs of a clam are reported in Table 2. Total lipid in a tissue ranged from 1 to 2.7% wet wt, in descending order of gonad, hepatopancreas, gill, mantle, and

TABLE 1. Average concentration (micrograms per gram wet weight) of the aromatic equivalent hydrocarbon in control and contaminated tissues of clams determined on 0.15-g homogenized samples.

Tissue	Control ($n = 2$)	Contaminated ($n = 3$)
Intestine[a]	27	1798
Hepatopancreas	27	399
Gonad	14	150
Gill	10	104
Muscles[b]	5	83
Mantle	5	68

[a] Midgut and rectum.

[b] Foot and adductor muscles.

TABLE 2. The relation of oil accumulation to the wet weight (C) and lipid content (F) of a tissue. Column E shows an estimation, based on 40 clams, of the amount of Kuwait crude oil that would be accumulated in an average clam of 5 g wet wt (without shell) under the experimental conditions. Intestine is excluded because of the difficulties in obtaining sufficient material.

Tissue	(A) Total wet wt in 40 clams (*g*)	(B) Estimated wet wt in an avg 5-g clam (*g*)	(C) Kuwait crude oil incorporated/g wet tissue (from Table 1) ($\mu g/g$)	(D) Lipid content/g wet tissue (*mg/g*)	(E = B × C) Oil accumulated in a 5-g clam (μg)	(F = C ÷ D) Kuwait crude oil/g lipid (*mg/g*)
Intestine	—	—	1771	—	—	—
Hepatopancreas	8.1	0.44	372	25.7	164	14.5
Gonad	23.4	1.23	136	26.5	168	5.1
Mantle	27.0	1.41	62	11.2	89	5.6
Muscle	18.5	0.97	78	10.0	76	7.8
Gill	17.8	0.93	94	17.0	88	5.5
					Total = 585	

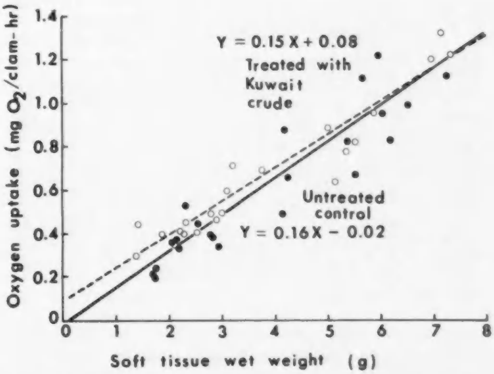

FIG. 2. Oxygen consumption by various sizes of *Mya arenaria* in uncontaminated sea water with and without previous exposure to seawater-accommodated Kuwait crude oil (concentrations between 39 and 128 μg/liter) for 30 days at 15 C. There are no significant differences (95%) in the slopes of regression lines and the intercept values calculated for clams of 4.20 g wet wt.

muscle. This probably represents the lowest concentration found during an annual cycle because the measurements were made in late summer just after the spawning season when most stored fats are utilized. My own observations in early summer indicate that the concentrations of storage lipids are higher in all tissues, ranging from 1.3 to 5.6% wet wt.

The relationship of oil accumulation to the wet weight and lipid content of a tissue has been calculated. Under the experimental conditions, the amount of Kuwait crude oil that would be accumulated in an average clam of 5 g wet wt (without shell) is about 6 mg (Column E, Table 2). The amount of Kuwait crude oil equivalent in a gram of lipid is approximately 6 mg for all tissues, with the exception of hepatopancreas and intestine (Column F, Table 2).

Respiration rates of gill tissue were compared by paired *t*-test. No significant changes ($P > 0.05$) from control times (0.009 mg O_2/h) could be detected after 1 h treatment with either 0.7 ($n = 6$) or 7 mg oil/liter ($n = 5$).

Oxygen consumption rates by clams of various sizes, after prolonged exposure to low concentration of Kuwait crude oil, were plotted against the wet weights (without shell) of respective clams. Regression lines were calculated with data from experimental and control clams, such that their slope and intercept values could be compared by a *t*-test. There was no significant difference in slopes of regression lines. Intercept values, calculated for clams of 4.20 g wet wt without shell, were not altered significantly from the control (Fig. 2).

However, when small clams were subjected to a higher concentration of oil in sea water at a slightly higher temperature than the animals in the previous experiment, a paired *t*-test showed a significant increase ($P < 0.95$) in oxygen uptake in clams exposed to Kuwait crude oil (Fig. 3). No information is available on the effect of such treatments on larger clams (>4.50 cm in shell length).

Discussion

UPTAKE AND RETENTION OF KUWAIT CRUDE OIL

The passage of petroleum hydrocarbons into shellfish has been presumed to take place by various routes. Lee et al. (1972) suggested trans-

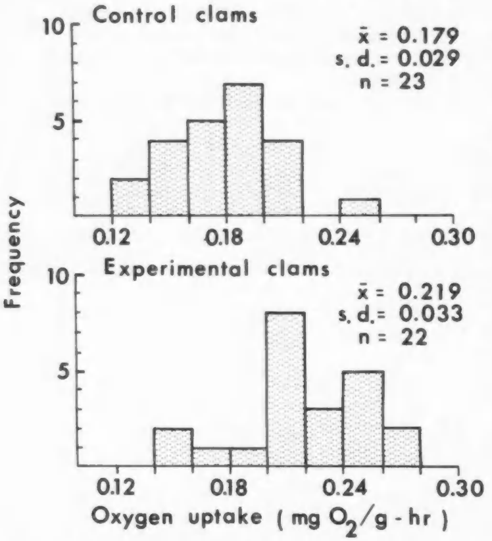

FIG. 3. Frequency distributions of the oxygen consumption by small clams in uncontaminated sea water with and without previous exposure to sea-water-accommodated Kuwait crude oil (concentrations between 220 and 370 μg/liter) for 21 days at 18 C. Shell length of experimental animals ranged from 3.82 to 4.44 cm, and the average soft tissue wet weight is 1.87 g. The corresponding values for control animals are 3.81–4.46 cm, and 1.94 g respectively. Difference significant at 95% level, $t = 3.70$, C.R. = 1.96.

port across outer membranes. Gill surfaces appear to be an important site of uptake. However, on prolonged exposure to oil, extremely high concentrations of oil are accumulated in the intestine and hepatopancreas (Table 1). This is to be expected because gill cilia treat oil globules as food particles, and particle-size distribution of oil globules in sea water coincides with the particle-size range retainable by clams. Dyed oil and dyed oil-mucus have been observed in the stomach and digestive diverticula of clams (Stainken 1975). Since the rate of uptake of oil must in part be determined in the concentration of oil at the absorbing surface (Hamelink et al. 1971; Stegeman and Teal 1973), oil uptake by the digestive tract may also be important.

Crude oils contain molecules of different size in fairly even distribution; organisms, on the other hand, favor the production of specific hydrocarbons (Blumer et al. 1970). This generalization applies to the aromatic fractions in Kuwait crude oil and *n*-hexane extracts of control clams, as clearly illustrated by the synchronous spectra in Fig. 1. The number of rings in the aromatic compounds producing the major peaks have been identified by Gordon and Keizer (1974). Thus, clams contain almost exclusively aromatic compounds of one and two rings. Detectable amounts of aromatic compounds with greater number of rings are found only in Kuwait crude oil and oil-contaminated clams. This difference in the amounts of aromatic compounds with a high number of rings in control and contaminated animals may be used as a criterion for the presence of petroleum hydrocarbons in animal tissues.

There are some limitations in the use of fluorescence spectroscopy for qualitative and quantitative determinations of oil in animal tissues (Gordon and Keizer 1974). Synchronous spectra of contaminated tissues do not reveal the exact nature of the compounds that cause differences in spectra when compared to controls. For example, the possibility that pollutants induce the formation of aromatic compounds in clams cannot be eliminated. However, this does not prevent the use of fluorescence spectroscopy to identify animals that have been exposed to oil. In my quantitative measurements with the fluorescence method, it was assumed that the aromatic and aliphatic fractions of the oil were taken up at equal rates. This is unlikely to be true. Stegeman and Teal (1973) observed that the petroleum hydrocarbons contained in oysters (*Crassostrea virginica*) differed from the contaminating oil by displaying a greater aromatic content presumably due to the greater water solubilities of aromatic compounds (McAuliffe 1966). The influence of the composition of the various lipid pools of clams, particularly membrane lipids, on the uptake of various fractions of oil is not known. Thus, the data presented in this paper are only rough estimates, but they provide reliable information on the relative abundance of oil in various contaminated tissues.

Blumer et al. (1970) have suggested that petroleum hydrocarbons taken up by marine organisms become part of their lipid pool. Accordingly there should be a relation between the lipid content of a tissue and the amount of oil that can be accumulated in the tissues. My results indicate that the hypothesis may be correct for clams. Approximately 6 mg of petroleum hydrocarbons/g lipid was accumulated in all tissues except hepatopancreas and intestine. These tissues perform digestive functions, thus, perhaps, complicating the comparisons by their content of ingested oil globules.

EFFECT OF KUWAIT CRUDE OIL ON THE RESPIRATION OF CLAMS

Changes in physiological activities of organisms may serve as indicators of sublethal effects of pollutants on organisms (Sprague 1971; Swedmark et al. 1971; Hargrave and Newcombe 1973). Of the three different experiments designed to measure sublethal effects only one demonstrated that Kuwait crude oil can cause significant increases in the respiratory rates of clams. Even these effects were only achieved in small clams (3.75–4.50 cm shell length) after prolonged exposure to relatively high concentrations of oil (220–370 μg/liter) and temperature (18 C). Short-term exposure to high concentrations (7 mg/liter) did not alter gill tissue respiration. Similarly, the respiration rates of clams of various sizes were not significantly affected after prolonged exposure to low concentrations of oil (Fig. 2). Other studies have also reported that oil of high concentrations can be harmful to clams. For example, Anderson (1972) reported that oil emulsions increased the filtration rate of *Mya arenaria*. Thomas (1973) observed extensive mortality in *M. arenaria*, following the oil spill (Bunker C crude oil) in Chedabucto Bay, Nova Scotia, in 1970. The feeding and assimilation rate of *M. arenaria* decreased by 50% on exposure to No. 6 fuel oil (E. S. Gilfillan and S. A. Hanson unpublished data).

For studies of sublethal effects of pollutants on organisms, it is helpful if animals are conditioned to their highest active physiological states for most sensitive measurements. An example would be to subject clams to an artificially severe stress by keeping them in moist air before returning to water for oxygen uptake measurements. Organisms in an active state will respond more readily to toxic substances than when in a resting state. Hence, Hargrave and Newcombe (1973) observed a measurable increase in oxygen uptake in intertidal *Littorina littorea* exposed to Bunker C oil at 18 C but not 4 C. The results of Gilfillan (1975) also clearly indicate that the action of salinity stress serves to enhance the effects of oil. Similarly, young organisms are more suitable for toxicity studies than adults because they have a higher metabolic rate per unit biomass. This is especially important when the pollutant under study is of low toxicity and applied in low concentrations.

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ANDERSON, G. E. 1972. The effects of oil on the gill filtration rate of *Mya arenaria*. Va. J. Sci. 23: 45–47.

BLIGH, E. G., AND W. J. DYER. 1959. A rapid method of total lipid extraction and purification. Can. J. Biochem. Physiol. 37: 911–917.

BLUMER, M., G. SOUZA, AND J. SASS. 1970. Hydrocarbon pollution of edible shellfish by an oil spill. Mar. Biol. 5: 195–202.

CAHNMANN, H. J., AND M. KURATSUNE. 1957. Determination of polycyclic aromatic hydrocarbons in oysters collected in polluted water. Anal. Chem. 29: 391–422.

COLLIP, J. B. 1921. A further study of the respiration processes in *Mya arenaria* and other marine mollusca. J. Biol. Chem. 49: 297–310.

DUNNING, A., AND C. W. MAJOR. 1974. The effect of cold seawater extracts of oil fractions upon the blue mussel, *Mytilus edulis*, p. 349–366. *In* F. J. Vernburg and W. B. Vernburg [ed.] Pollution and physiology of marine organisms. Academic Press, Inc., New York, N.Y.

EHRHARDT, M. 1972. Petroleum hydrocarbons in oysters from Galveston Bay. Environ. Pollut. 3: 257–271.

FOLCH, J., M. LEE, AND G. H. S. STANLEY. 1957. A simple method for the isolation and purification of total lipids from animal tissues. J. Biol. Chem. 226: 497–509.

GILFILLAN, E. S. 1975. Decrease of net carbon flux in two species of mussels caused by extracts of crude oil. Mar. Biol. 29: 53–57.

GORDON, D. C. JR., AND P. D. KEIZER. 1974. Estimation of petroleum hydrocarbons in sea water by fluorescence spectroscopy: improved sampling and analytical methods. Fish. Mar. Serv. Res. Dev. Tech. Rep. 481: 28 p.

HAMELINK, J. L., R. C. WAYBRANT, AND R. C. BALL. 1971. A proposal: exchange equilibria control the degree chlorinated hydrocarbons are biologically magnified in lentic environments. Trans. Am. Fish. Soc. 100: 207–214.

HARGRAVE, B. T., AND C. P. NEWCOMBE. 1973. Crawling and respiration as indices of sublethal effects of oil and a dispersant on an intertidal snail *Littorina littorea*. J. Fish. Res. Board Can. 30: 1789–1792.

HARGRAVE, B. T., AND G. A. PHILLIPS. 1975. Estimates of oil in aquatic sediments by fluorescence spectroscopy. Environ. Pollut. 8: 193–215.

KEIZER, P. D., AND D. C. GORDON JR. 1973. Detection of trace amounts of oil in sea water by fluorescence spectroscopy. J. Fish. Res. Board Can. 30: 1039–1046.

LEE, R. F., R. SAUERHEBER, AND A. A. BENSON. 1972. Petroleum hydrocarbons: uptake and discharge by the marine mussel *Mytilus edulis*. Science 177: 344–346.

LLOYD, J. B. F. 1971. The nature and evidential value of the luminescence of automobile engine oils and related materials. Part I. Synchronous excitation of fluorescence emission. J. Forensic Sci. Soc. 11: 83–94.

MCAULIFFE, C. D. 1966. Solubility in water of paraffin, olefin, acetylene, cycloolefin, and aromatic hydrocarbons. J. Phys. Chem. 70: 1267–1275.

SPRAGUE, J. B. 1971. Measurement of pollutant toxicity to fish. III. Sublethal effects and 'safe' concentrations. Water Res. 5: 245–266.

STAINKEN, D. M. 1975. Preliminary observation on the mode of accumulation of #2 fuel oil by the soft shell clam, *Mya arenaria*, p. 463–468. *In* Proc. Conf. Prev. Control Oil Spills, San Francisco, Calif., 1975. Pet. Inst., New York, N.Y.

STEGEMAN, J. J., AND J. M. TEAL. 1973. Accumulation, release and retention of petroleum hydrocarbons by the oyster (*Crassostrea virginica*). Mar. Biol. 22: 37–44.

SWEDMARK, M., B. BRAATEN, E. EMANUELSSON, AND A. GRANMO. 1971. Biological effects of surface active agents on marine animals. Mar. Biol. 9: 183–201.

THOMAS, M. L. H. 1973. Effect of Bunker C oil on intertidal and lagoonal biota in Chedabucto Bay, Nova Scotia. J. Fish. Res. Board Can. 30: 83–90.

VAHL, O. 1972a. Efficiency of particle retention in *Mytilus edulis* (L). Ophelia 10: 17–25.

1972b. Particle retention and relation between water transport and oxygen uptake in *Chlamys opercularis* (L) (Bivalvia). Ophelia 10: 67–74.

1973. Efficiency of particle retention in *Chlamys islandica* (O. F. Muller). Astarte 6: 21–25.

VAN DAM, L. 1935. On the utilization of oxygen by *Mya arenaria*. J. Exp. Biol. 12: 80–85.

High Seas Oil Pollution: Particulate Petroleum Residues in the North Atlantic[1]

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LEVY, E. M., AND A. WALTON. 1976. High seas oil pollution: particulate petroleum residues in the North Atlantic. J. Fish. Res. Board Can. 33: 2781–2791.

The results of a continuing investigation into the occurrence and distribution of particulate petroleum residues on the surface of the North Atlantic are presented. From 1971 to 1974 more than 850 samples were collected from the North Atlantic on transects between the east coast of Canada and South America, the Caribbean, Baffin Bay and surrounding waters, the Labrador Sea, and the Azores. Repeated sampling was carried out in the Gulf of St. Lawrence and the region between Nova Scotia and Bermuda. The results indicate that the waters north of the Gulf Stream–North Atlantic Current system were virtually free from floating petroleum residues, while the waters of the Gulf Stream, Sargasso Sea, and Caribbean Sea were much more heavily polluted. Although concentrations as high as 91.8 mg/m^2 were encountered, the general level of pollution was much less with tar contents in southern waters following a lognormal distribution with a geometric mean of 0.16 mg/m^2. The geographical distribution of tar is interpreted in terms of inputs from shipping and tanker traffic, and surface circulation patterns.

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Nous donnons ici les résultats d'une étude continue de l'occurrence et de la répartition de résidus particulaires de pétrole à la surface de l'Atlantique Nord. Entre 1971 et 1974, plus de 850 échantillons ont été recueillis en Atlantique Nord sur des lignes allant de la côte orientale du Canada à l'Amérique du Sud, les Caraïbes, la baie de Baffin et les environs, la mer du Labrador et les Açores. Un échantillonnage répété a été effectué dans le golfe Saint-Laurent et la région allant de la Nouvelle-Ecosse aux Bermudes. Les résultats indiquent que les eaux situées au nord du réseau Gulf Stream/courant de l'Atlantique Nord sont pratiquement libres de résidus de pétrole flottants, alors que les eaux du Gulf Stream, de la mer des Sargasses et de la mer des Caraïbes sont beaucoup plus polluées. Bien que nous ayons rencontré des concentrations aussi élevées que 91.8 mg/m^2, le niveau général de pollution est beaucoup moins élevé quant au contenu en goudron dans les eaux méridionales, qui ont une distribution lognormale d'une moyenne géométrique de 0.16 mg/m^2. Nous interprétons la répartition géographique du goudron en termes de transports maritimes et de trafic de pétroliers, et de circulation de surface dans l'océan.

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HEYERDAHL (1971), in accounts of his voyages on *Ra* and *Ra II*, has vividly drawn attention to the existing oil contamination of the Atlantic Ocean. Recent studies suggest that approximately 5.5 million metric tons/yr of petroleum hydrocarbons are entering the world's oceans as a direct consequence of human activity, the major contributor being marine transportation (Anon. 1975). In the oceans, oil is exposed to a variety of biological, chemical, physical, and geological processes that alter its chemical composition and its physical form. Some fractions congeal into lumps, popularly known as "tar balls." These residues degrade slowly and may be transported for great distances over the ocean under the influence of winds and surface currents.

The presence of particulate petroleum residues on the surface of the open ocean was first reported in the scientific literature by David (1965). At the time tar particles were considered as something of an oddity as they provided an unusual floating substrate for barnacles. A few years later, however, oil pollution was sufficiently abundant in some areas that it presented serious problems to studies of surface fauna (Blumer 1969). The first semiquantitative measurements of tar in the North Atlantic were reported by Horn et al. (1970) who observed lumps ranging in size from 1 or 2 mm to 10 cm in 75% of 734

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[1] Bedford Institute of Oceanography Contribution.

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neuston tows from the Mediterranean and the North Atlantic between Gibraltar and the Azores in 1969. Concentrations reached 540 mg/m^2 in the Mediterranean between Libya and Sicily, while much lower concentrations, 5 mg/m^2 or less, were found in the western basin of the Mediterranean and in the eastern North Atlantic. In the western North Atlantic, concentrations ranging from 0.1 to 9.7 mg/m^2 were observed by Morris (1971) at 5 stations along the edge of the Scotian Shelf in 1969 and at 14 stations south of the Grand Banks and one near Bermuda in 1970. Although these observations demonstrated the occurrence of tar in the North Atlantic, there were not sufficient data available to permit meaningful calculations of the amount of tar present or to relate its distribution to anticipated inputs and the oceanographic features of the region. The present study was initiated to provide these necessary data.

Materials and methods

SAMPLING

To obtain as many samples as possible in this program, advantage was taken of research vessels and ships with scientifically oriented personnel. Consequently, it was essential that the sampling procedures be simple, convenient, and capable of being carried out without unduly hindering the progress of the ship. A modified version of a neuston sampler, originally described by Sameoto and Jaroszynski (1969), met these requirements and also performed satisfactorily in all but severe weather conditions (Levy and Walton 1971). It was towed for 1 nautical mi (1.9 km) at a speed of 5–7 knots (2.5–3.5 m/s) to sample approximately 740 m^2 of sea surface. In regions of high biological productivity, sampling was restricted to daylight hours to avoid voluminous catches of organisms. At the end of the towing period, the sampler was recovered and its contents were transferred to a glass jar and immediately deep frozen to prevent bacterial alteration.

In addition to tar lumps, samples collected on the high seas by this method frequently contained a miscellany of seaweed, phyto- and zooplankton, nekton, small fish, terrestrial insects, feathers, bits of wood and tree bark, as well as paint chips, shreds of plastic, spherules of polyethylene, beads or chunks of styrofoam, and other floating debris. In the event that an excessive amount of this extraneous material was also collected, the combined materials were rinsed in a container of sea water and the tar balls recovered by filtering the water through the net. If the sampler or net became fouled with fresh, sticky oil, the soiled areas were cleaned with carbon tetrachloride or other solvent and the washings retained for analysis.

PROCESSING AND ANALYSIS OF SAMPLES

After the frozen sample had melted, the tar balls were separated manually from extraneous material. If a large amount of tar was involved (about 25 samples contained 0.5 g or more), it was air-dried and weighed directly. Since samples from the high seas seldom contained sand, the presence of small amounts of extraneous materials did not give rise to serious errors in these samples. In the remaining cases, the following extraction procedure was used. After physical separation the tar mixture was extracted with successive aliquots of carbon tetrachloride until no further discoloration of clean solvent occurred. The tar particles that had been handpicked from the mixture were then added to the carbon tetrachloride extract and dissolved. The residue, after evaporation of the carbon tetrachloride, was similarly extracted with *n*-hexane and centrifuged. The *n*-hexane insoluble residue was then extracted with benzene. After evaporation of the solvents the weights of the hexane- and benzene-soluble fractions were obtained, and the sum of these two fractions was considered as the total weight of the tar in the sample.

Although some volatile components of the tar would be lost during the evaporation processes, this is not a major source of error since most of the lower boiling components would have already been lost by weathering. On the other hand, the procedure effectively eliminated water and inorganic materials which would otherwise have led to erroneously high values in the small weights of tar generally involved. The presence of such substances is a more significant source of error than losses of small amounts of volatile substances.

AREAS SURVEYED AND NUMBER OF SAMPLES COLLECTED

From 1971 to 1974, 874 surface samples were collected from the North Atlantic and its bordering seas (Fig. 1). Sampling was carried out from the coast of South America to the limit of marine navigation in the arctic (about 78°N) and from the east coast of North America to Europe and into the Mediterranean Sea. Coverage was somewhat irregular with some areas of the North Atlantic sampled comparatively densely and on a repeated basis, while others were sampled only sparsely. Unfortunately, large areas, particularly in the eastern and central regions, remain to be sampled. The Gulf of St. Lawrence and the region between Nova Scotia and Bermuda have been sampled on an annual basis, or more frequently.

Results and Discussion

DESCRIPTION OF SAMPLES

The tar lumps varied greatly in size, texture, and appearance. The vast majority were < 10 mm diam, while in a few cases tar balls > 1–2 cm diam and, in rare instances, lumps about the size of golf balls or larger were encountered. The consistency of the samples ranged from that of fresh, sticky, semifluid oil to relatively hard, nonsticky, and almost granular lumps. Some had shiny surfaces, indicating relatively fresh or only

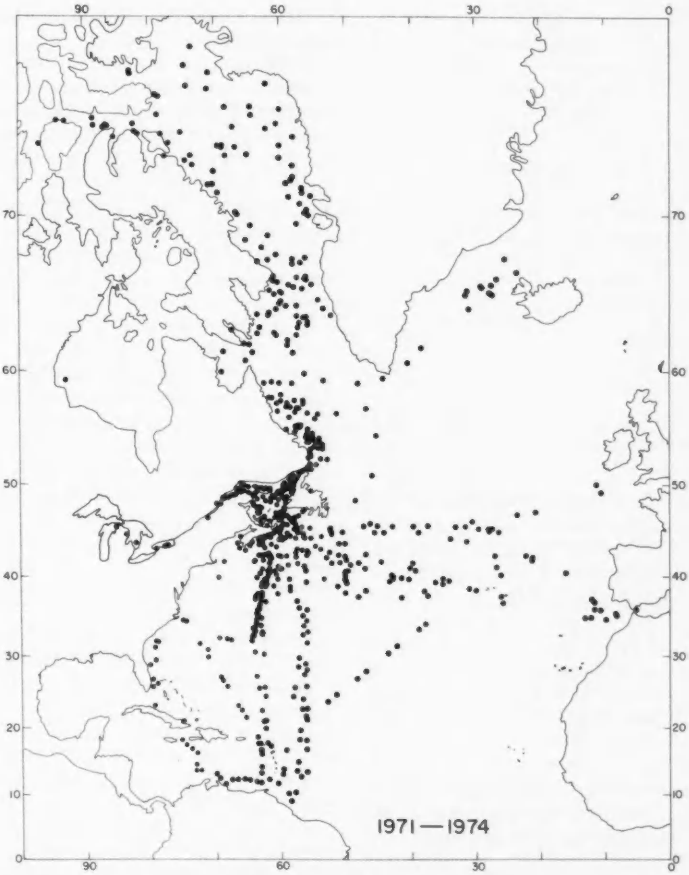

FIG. 1. Locations from which samples were collected 1971–74 inclusive.

partially weathered oil, while others dull surfaces, suggesting coatings of algae, fungi, bacteria, or other microorganisms. Presumably the outward appearance of the tar particles reflected their past history, i.e. the diverse origins of the oil and the changes that occurred during its exposure to the marine environment. In some instances, weathering appeared to have proceeded to an advanced stage as the particles were crumbly and had an outward appearance suggestive of soil particles.

OCCURRENCE OF PARTICULATE PETROLEUM RESIDUES IN THE NORTH ATLANTIC

The occurrence of tar in all samples is indicated by the histograms in Fig. 2, while details of the original data for 1971–74 are given elsewhere (Levy and Moffatt 1975). More than 50% of the samples collected between early 1971 and the end of 1974 did not contain sufficient tar for detection by the techniques employed (detection limit: approximately 0.1 $\mu g/m^2$) and of those that did, most contained < 1 mg/m^2. A substantial number (about 9%) of the samples, however, contained tar in excess of this value with one exceptionally rich sample from the Sargasso Sea in 1973 containing 91.8 mg/m^2. Overall, 874 samples, having an average tar content of 0.57 $mg/m^2 \pm 3.64$ SD were collected.

The average annual concentrations are included in Fig. 2. Statistical examination (*t*-tests) of the data indicate that only the increase between 1971 and 1972 is mathematically significant. This increase is believed to be a consequence of the fact that a greater portion of the samples during 1972 were collected from the southern portion of the survey area where tar

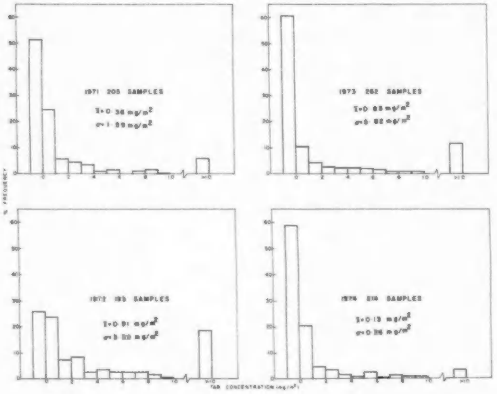

FIG. 2. Annual frequency distribution plots including arithmetic means and standard deviations.

was more commonly encountered than in the northern regions, rather than an indication of an actual change in the overall level of pollution. This supposition is supported by a comparison of the numbers of samples containing no evidence of tar, namely 51.2% in 1971, 25.9% in 1972, 60.7% in 1973, and 58.9% in 1974. It is difficult, therefore, to confirm from these data either a significant increase or decrease in the level of tar pollution in the North Atlantic during the study.

The high standard deviations (Fig. 2) reflect the patchiness of the distribution of the tar present on the surface of the North Atlantic. Such variability clearly demonstrates the pitfalls associated with attributing any great significance to data obtained from the occasional, isolated sampling location or even from repeated sampling in a very restricted area of the ocean. Therefore, it is necessary to carry out an appropriate statistical analysis of the data collected over a broad expanse of the ocean and over a prolonged period of time before a reliable estimate of the total amount of tar present and its distribution can be made.

The most striking general feature of the data, however, is that nearly all of the samples that did not contain tar were collected in the region to the north and west of the Gulf Stream–North Atlantic Current systems. Because of this geographical separation, the samples may be considered as belonging to two distinct populations, those that contained measurable amounts of tar and those that did not. On this basis, then, the average tar concentration in the northern region can be considered zero, while in southern waters nonzero (Table 1). Statistical examinations again demonstrated that the observed differences in the average annual tar contents were not mathematically significant.

Although the arithmetic mean and standard deviation are appropriate statistics for normally distributed populations, highly skewed distributions such as those in Fig. 2 are better treated by lognormal methods (Aitchison and Brown 1969). As shown in Fig. 3 and further confirmed by chi-square tests, the logarithms of the tar contents were normally distributed for those samples that contained sufficient tar for detection. The distribution of particulate petroleum residues thus appears to be analogous to the distribution of mineral in an ore body where most of the desired mineral is associated with only a portion of the body, some parts of which are exceptionally rich, while the overall average for the body is much smaller and surrounding areas are devoid of the desired mineral. The tar in the North Atlantic indicates a similar irregular pattern with a more or less general level of pollution over a broad expanse of southern waters, where there are limited areas with high levels of contamination, while in the northern waters pollution is virtually nonexistent or, at most, restricted to isolated localities.

Using the two-box model proposed in the foregoing, the average annual concentrations of tar in the southern waters were calculated from the log-transformed data (Table 2). Mathematically significant differences were found between 1971 and 1972 and between 1973 and 1974 but not between 1972 and 1973. Differences between the means were also significant for each of the individual years except 1972 as were those for the combined data for the entire period of the study. Thus, there appear to be significant changes in central tendency that are evident in the log-transformed data that were not detected by the arithmetic means. Nevertheless, while these differences are mathematically significant, it is not possible to say whether they indicate true temporal changes in the level of pollution in the North Atlantic or merely reflect the patchiness of its geographical distribution combined with differences arising from regional variations in sampling from year to year.

AMOUNT OF TAR IN THE NORTH ATLANTIC

Assuming the waters north of 45°N to be essentially free from tar pollution and using the mean concentrations observed during each of the years 1971–74 along with their corresponding 95% confidence intervals (Table 2), tar loadings for the North Atlantic were calculated (Table 3). Whereas the range of values for the individual

TABLE 1. Concentration of tar observed in the southern regions of the North Atlantic.

	1971	1972	1973	1974	1971–74
No. of samples	100	143	103	88	434
Avg concn (*mg*/*m*²)	0.73	1.23	2.17	0.32	1.16
±SD (*mg*/*m*²)	2.22	3.79	9.16	0.51	5.11

TABLE 2. Concentration of tar in the southern regions of the North Atlantic (log-transformed data).

	1971	1972	1973	1974	1971–74
No. of samples	100	143	103	88	434
Avg log concn	−1.123	−0.642	−0.480	−1.036	−0.795
± SD	1.005	0.875	0.939	0.775	0.937
Geometric mean (*mg*/*m*²)	0.0753	0.228	0.331	0.092	0.160
95% Confidence interval					
−Log concn	1.31–0.93	0.79–0.50	0.66–0.30	1.20–0.87	0.88–0.71
Geometric mean (*mg*/*m*²)	0.048–0.12	0.16–0.32	0.22–0.50	0.063–0.13	0.13–0.20

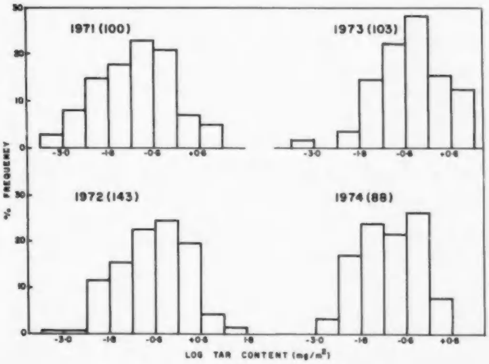

FIG. 3. Annual frequency distributions-log transformed data.

TABLE 3. Estimates of the total amount of tar in the North Atlantic.

Year	95% confidence interval (10³ *metric tons*)	Most likely value (10³ *metric tons*)
1971	1.55–3.83	2.43
1972	5.29–10.25	7.36
1973	7.04–16.24	10.7
1974	2.05–4.32	2.97
1971–74	4.23–6.34	5.17

years extended from 1,600 to 16,200 metric tons, the overall tar burden for the entire period was somewhere between 4,200 and 6,300 metric tons with the most probable value being about 5,200 metric tons. (Considerably higher values would have been obtained if the arithmetic rather than the geometric mean had been used.)

A number of other estimates of the total amount of tar in the North Atlantic have been made previously. Morris (1971), for example, first estimated that there were 27,000 tons of tar in the North Atlantic on the basis of a geometric mean of 0.93 mg/m² for 20 samples collected from the Scotian Shelf, Gulf Stream, and Bermuda areas. Subsequently, this value was revised after extensive sampling at one location in the Sargasso Sea near Bermuda and it was then estimated that there were 2,000 tons of tar in the Northwest Atlantic Marginal Sea (Scotian Shelf), 18,000 tons in the Gulf Stream, and 70,000 tons in the Sargasso Sea (Morris and Butler 1973). Mean concentrations of 0.2, 0.6, and 0.8 mg/m² (not indicated whether arithmetic or geometric) for the U.S. east coast continental shelf (K. J. Sherman et al. unpublished data; D. Attaway et al. unpublished data), the Caribbean Sea and the Gulf of Mexico (L. M. Jeffrey unpublished data; K. J. Sherman et al. unpublished data) were recently used to derive estimates of tar loadings of 200, 1,200, and 1,600 tons for each of these regions (Anon. 1975). The average tar concentrations reported for the U.S. east coast continental shelf area are in good agreement with the values observed during this study, and in addition, our data with concentrations observed by the U.S. Coast Guard at the Ocean Weather stations in the North Atlantic (McGowan et al. 1974).

Since the estimates by Morris and Butler (1973) are based on a small number of measurements near the Gulf Stream and a series of measurements made at one locality in the Sargasso Sea some 32 km from Bermuda, their findings are strongly biased by the higher-than-average concentrations of tar that were repeatedly observed in that general area during the present study. Such concentrations do not appear to be typical of the Sargasso Sea as a whole, and thus, we believe that their overall estimates are probably high. Since the estimates made in the present study are based on a more extensive areal coverage of the North Atlantic, they are therefore more representative of the actual degree of pollution that exists in the North Atlantic.

SURFACE CIRCULATION AND THE GEOGRAPHICAL DISTRIBUTION OF TAR IN THE NORTH ATLANTIC

The geographical distributions of tar found on the surface of the North Atlantic during 1971–74 are illustrated by the contour maps in Fig. 4–7 where it is apparent that tar was only rarely encountered in waters north of about 45°N lat., presumably because these regions are removed from major population centers and the main stream of tanker and other ship traffic, and the activities associated with the petroleum industry. In the more remote northern regions, the occurrence of tar was generally associated with the recorded passage of a ship through the area, and usually the tar was in a fresh, relatively unweathered form in contrast with the weathered material which was almost ubiquitous in the southern waters.

Equally striking were the high concentrations observed each year in the western Sargasso Sea. Tar concentrations tended to decrease outward from this region in a manner very closely related to the major surface currents in the western North Atlantic. The dominant feature of the surface circulation in the North Atlantic is the large clockwise gyre that occupies much of the region between 15 and 45°N (Fig. 8). The inner portion of this area, the Sargasso Sea, appears to be an effective "trap" for a considerable portion of the tar floating in the North Atlantic. Petroleum residues are probably transported out of the Sargasso Sea by the western boundary current, thus leading to contamination of the Gulf Stream and more northerly latitudes. On the other hand, petroleum concentrations decreased abruptly in the waters to the west and north of the Gulf Stream, and the southerly flow over the continental shelf of cold Labrador Current-dominated water maintained a much lower degree of pollution in the coastal waters off eastern Canada and the northeastern United States than that present in the Gulf Stream. Indeed, it was repeatedly demonstrated during this study that, on proceeding south from Nova Scotia, a marked and abrupt increase in tar concentration was

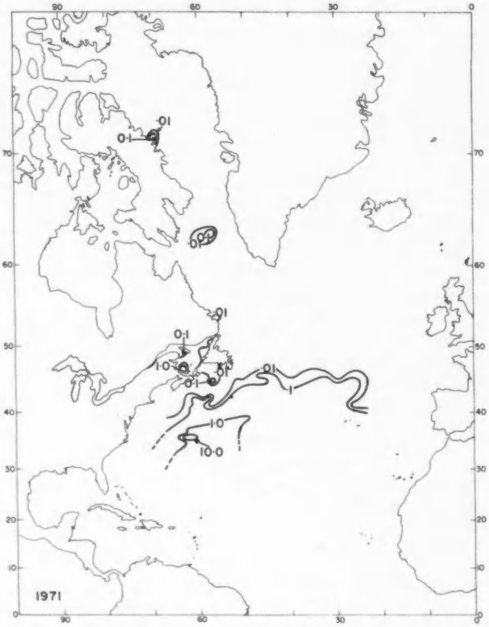

FIG. 4. Geographical distribution of tar on the North Atlantic, 1971.

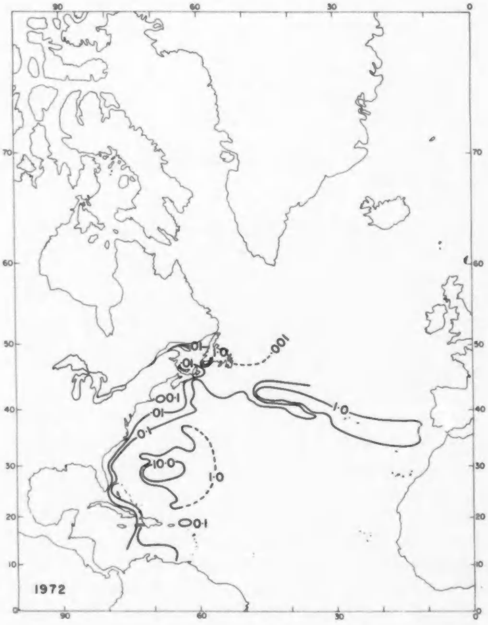

FIG. 5. Geographical distribution of tar on the North Atlantic, 1972.

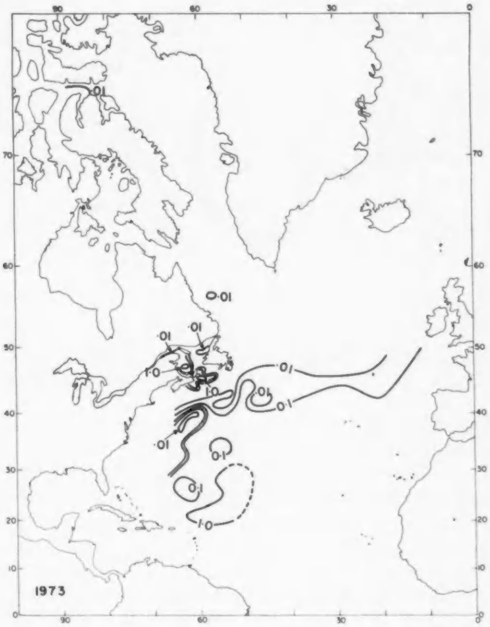

FIG. 6. Geographical distribution of tar on the North Atlantic, 1973.

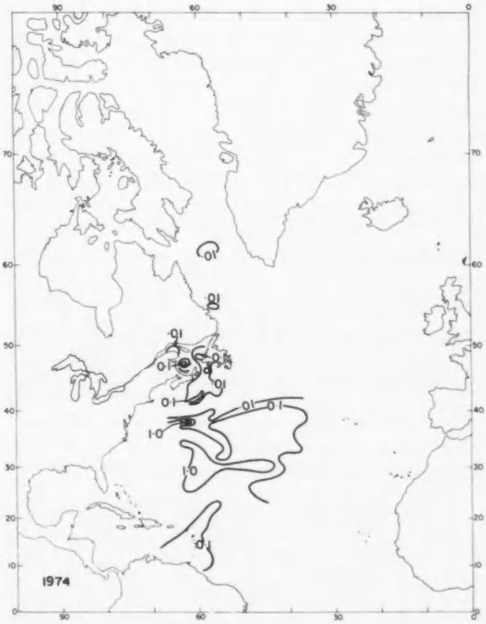

FIG. 7. Geographical distribution of tar on the North Atlantic, 1974.

always associated with the corresponding abrupt increase in temperature which signified that the boundary of the Gulf Stream had been reached.

The distribution of tar concentrations yields a much more complex pattern as the Gulf Stream flows northward and becomes less well defined. South of Newfoundland the current assumes a more easterly direction, presumably transporting its burden of tar with it across the Atlantic. Although our study did not provide opportunity to study the eastern portion of the North Atlantic gyre in detail, Ehrhardt and Derenbach (1975) recently observed concentrations of 0.007–20.5 mg/m^2 in 22 samples collected along a transect between Portugal and the Canary Current. In addition, Heyerdahl's (1971) description of the oil pollution in the Canary and North Equatorial currents leaves little doubt of the extent of the pollution in those regions. Although the origin of the petroleum pollution found in the various regions of the North Atlantic gyre will remain in doubt until an unambiguous method of source identification is found, tar could be transported from the western portions of the North Atlantic to the eastern zones and vice versa by the surface currents.

To the north of the gyre, areas sampled during the study were essentially free from pollution by floating tar. For example, the Labrador Sea and the waters which flow southward along the east coast of Greenland entering Davis Strait along the Greenland side and exiting on the Canadian side as the Labrador Current were found to be free from floating petroleum residues except for a few very restricted localities. Also free from pollution were the waters of Baffin Bay and those waters exiting from the Canadian archipelago. In these areas the only evidence of oil was in the vicinity of ports or areas through which ships had recently passed.

From the data collected so far in this study, the pollution of the North Atlantic by tar and floating oil thus appears to be associated primarily with the Sargasso Sea and the Gulf Stream system. Since other observations indicate that comparable, or even higher, levels of surface pollution are present in the Canary and North Equatorial currents, we may conclude that most, if not all, of the North Atlantic gyre is affected. Since the more resistant portions of the tar degrade slowly, the residual portions could make one or even more complete circuits around the gyre before weathering processes reached the stage where the residues disappeared completely from the surface of the ocean. In addition, oil released or spilled as far away as the Cape of Good Hope region might be carried by the

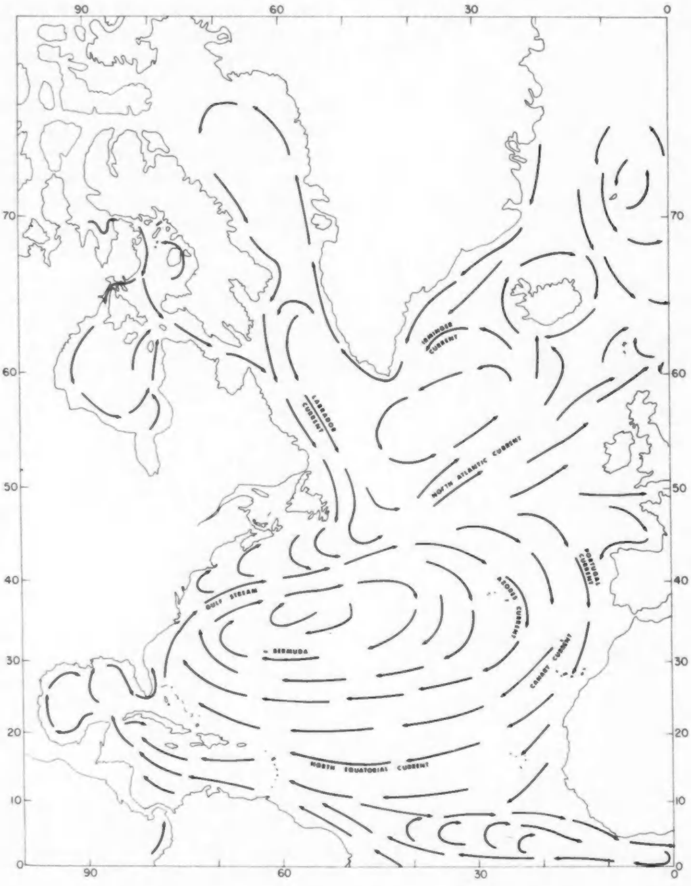
90
60
30
0
70
60
50
40
30
20
10
0
IRMINGER CURRENT
LABRADOR CURRENT
NORTH ATLANTIC CURRENT
GULF STREAM
BERMUDA
AZORES CURRENT
PORTUGAL CURRENT
CANARY CURRENT
NORTH EQUATORIAL CURRENT

FIG. 8. Major surface currents of the North Atlantic.

Benguela Current northwards and thence westward across the Atlantic by the South Equatorial Current, eventually entering the North Atlantic gyre. Further, the Atlantic to the west of Africa and the corresponding gyre in the South Atlantic might contain considerable amounts of petroleum residues as a result of the large volume of oil being transported along the Persian Gulf–Europe route.

The concentrations observed in this study and their strong association with the current systems in the North Atlantic are further supported by the U.S. Coast Guard observations of 0–0.003, 0–1.83, 0–10.73, and 0–21.62 mg/m^2 at the North Atlantic Ocean Weather stations, Bravo, Charlie, Delta and Echo, respectively, during 1971–73 (McGowan et al. 1974). In the present study a marked increase was observed from the essentially unpolluted waters in the north between Greenland and Labrador (average concentration 0.00 mg/m^2) to progressively higher levels in regions dominated by the North Atlantic Current and Gulf Stream (0.12 and 1.15 mg/m^2), with the highest levels of pollution in the Sargasso Sea (2.64 mg/m^2).

PETROLEUM RESIDUES AND TANKER TRAFFIC

It is perhaps not entirely coincidental that the highest levels of tar pollution in the North Atlantic were associated with these current systems. As indicated in Fig. 9, the major flow of petroleum through the North Atlantic takes place in a south–north direction from the producing areas of Venezuela and the Middle East to consumers in North America and Europe, although

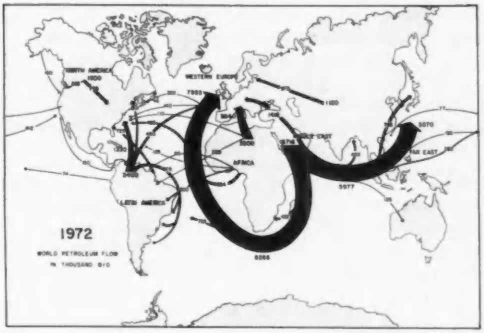

FIG. 9. World flow of oil (from International Petroleum Encyclopedia 1973).

there is a comparatively much smaller movement of oil across the Atlantic. Some 2.7 million barrels of oil/day (1 barrel/day = 0.136 metric tons/day) passed through the western portion of the survey area in 1971, while more than 7.8 million barrels/day flowed through the eastern portion (neglecting the south–north vector of oil transported across the Atlantic), while the corresponding values forecast for 1975 were 3.5 and 12.7 million barrels/day, respectively (Anon. 1973). Consequently, more extensive pollution might exist in the eastern portion of the North Atlantic in view of the much greater volume of tanker traffic there. Indeed, Monaghan et al. (1974) observed considerably higher concentrations of dissolved petroleum-derived substances in the waters along the tanker lanes between the Persian Gulf and Europe than those between the Caribbean and the east coast of North America and unequivocally attributed the higher concentrations to the much more extensive tanker traffic.

Although it is impossible to estimate with certainty the rate at which tar enters the North Atlantic, some estimates can be made on the basis of either a) the fraction of the total world flow of oil that passes through the North Atlantic or, alternatively, b) on the fraction of the total world consumption by those countries that border the North Atlantic.

a) The flow of petroleum through the North Atlantic during 1971 is estimated to have been 11.7 million barrels/day while that projected for 1975 is 19.8 million barrels/day (Anon. 1973). Assuming a linear increase in the flow of petroleum over this 5-yr period, the amounts of oil passing through the North Atlantic during each year of the survey and the total world flow of oil were calculated. (Although the increase in petroleum consumption has been more closely exponential, it is reasonable to assume linearity over a short period of time.) Since the best estimate of the total input of oil from all sources to the world oceans in 1973 was considered to be 6.1 million tons (Anon. 1975), it follows that on a world wide basis the average input was 0.38% of the total flow. If it is assumed that this percentage did not change over the course of our investigation, the amount entering the world ocean each year is readily calculated. Assuming further that the amount entering the North Atlantic is proportional to the amount of oil passing through the North Atlantic, the annual entry to the North Atlantic from 1971 to 1974 ranged from 2.2 to 3.4 million tons. The assumption that the amount of oil entering the North Atlantic is proportional to the amount flowing through it is reasonable since most, if not all, of this oil is utilized by the countries bordering the North Atlantic.

b) Using consumption data (Anon. 1973) and the estimate of 6.1 million tons of oil entering the world ocean in 1973, estimates of the input of tar to the North Atlantic range from 3.1 to 3.8 million tons for 1971–74 (Table 4). Although these estimates are somewhat higher than those based on the flow of oil, the general picture is similar. However, conservation measures and economies in the production, transportation, refining, and consumption of oil may succeed in reducing the amount lost to the ocean and by the early 1980s the input is estimated at 1.5–2.5 million tons/yr (Anon. 1975).

RESIDENCE TIMES

Although the fraction of the original input of oil that persists long enough to become transformed into tar differs for oils from different origins, as a first approximation it is postulated that about 1% of the total input provides the "feedstock" for the production of tar. (Elliott et al. [1972] have shown that 23–38% of two crude oils were lost by evaporation alone in less than a month and that 50% of this was lost in the first 5 h.) In this event, the input of tar to the North Atlantic would range from 22,000 tons in 1971 to 34,000 tons in 1974 using transportation data (Table 5) or 31,000–38,000 tons if the estimate is based on consumption data (Table 4). However resistant it may be, tar itself is not immune to weathering and has a limited lifetime on the surface of the ocean. The average amount of tar present in the North Atlantic calculated on the basis of the mean concentration observed in this study was 5,200 tons for 1971–74 (Table 1). On the basis of the average of the two estimates for the input for 1973, the "residence time" of tar in the North Atlantic would be about 0.16 yr or about 58 days.

TABLE 4. Estimate of tar input to North Atlantic based on consumption data.

Year	Consumption (10^6 *tons*) North America	Europe	Combined	World consumption (10^6 *tons*)	Input to ocean (10^6 *tons*) North Atlantic	World[b]	Entry of tar[a] into North Atlantic (*tons*)
1971	769	628	1397	2289	3.1	5.1	32,000
1972	829	670	1499	2518	3.3	5.6	33,000
1973	889	712	1601	2748	3.6	6.1	36,000
1974	949	754	1703	2977	3.8	6.6	38,000

[a] Based on 1% converted to tar.
[b] Based on (6.11/2748) × 100 = 0.22% loss.

TABLE 5. Estimate of tar input to North Atlantic based on transportation data.

Year	Flow through North Atlantic (10^6 *tons*)	Total world flow (10^6 *tons*)	Entry[a] of oil to North Atlantic (10^6 *tons*)	World ocean (10^6 *tons*)	Entry[b] of tar to North Atlantic (*tons*)
1971	586	1,270	2.2	4.8	22,000
1972	688	1,437	2.6	5.5	26,000
1973	789	1,604	3.0	6.1	30,000
1974	891	1,771	3.4	6.7	34,000

[a] Based on (6.11/1604) × 100 = 0.38% rate of loss for 1973.
[b] Based on 1% converted to tar.

Conclusions

This study provides an overview of the distribution of tar on the surface of the North Atlantic and how the distribution is related to surface circulation and expected inputs. Superimposed upon this general overview are many perturbations arising from highly variable and transient local and seasonal phenomena. While it is appreciated that there are still many gaps to be filled before anything approaching a complete understanding of high seas oil pollution is attained, the present study accounts for at least the gross features of the distribution of tar in the North Atlantic and is a first step towards this goal.

Acknowledgments

A large number of scientists and technicians, ship's officers and crew participated in this program by collecting samples. Without their cooperation this study would not have been possible, and we acknowledge with gratitude the very fine efforts these people have made at sea. In the laboratory, L. R. Webber and J. D. Moffatt analyzed the samples while J. L. Barron prepared the computer programs and C. C. Cunningham the illustrations. To all we are extremely grateful.

AITCHISON, J., AND J. A. C. BROWN. 1969. The lognormal distribution. Cambridge University Press, New York, N.Y. 176 p.

ANON. 1973. International petroleum encyclopedia. The Petroleum Publishing Co., Tulsa, Okla. 440 p.

1975. Petroleum in the marine environment. Workshop on inputs, fates and effects of petroleum in the marine environment. Natl. Acad. Sci., Washington, D.C. 228 p.

BLUMER, M. 1969. Oil pollution of the ocean. Oceanus 15: 2–7.

DAVID, P. M. 1965. The surface fauna of the ocean. Endeavour 24: 95–100.

EHRHARDT, M., AND J. DERENBACH. 1975. Abstract submitted to ICES workshop on petroleum hydrocarbons in the marine environment. Int. Counc. Explor. Sea (ICES), Aberdeen, Scotland.

ELLIOTT, J. J., T. D. SEARL, AND R. A. BROWN. 1972. The measurement of hydrocarbons in ocean water — the IR absorptivities of total and weathered crudes and of petroleum distillates. Append. E in Monaghan et al. (1974)

HEYERDAHL, T. 1971. The Ra expeditions. Doubleday and Company, Inc., Garden City, N.Y.

HORN, M. J., J. M. TEAL, AND R. H. BACKUS. 1970. Petroleum lumps on the surface of the sea. Science 168: 245–246.

LEVY, E. M., AND J. D. MOFFATT. 1975. Data report: floating petroleum residue concentrations in the North Atlantic 1971–1974. Bedford Inst. Oceanogr., Atl. Oceanogr. Lab. Rep. BI-D-75-9: 56 p.

LEVY, E. M., AND A. WALTON. 1971. An evaluation of two neuston samplers. Bedford Inst. Oceanogr., Atl. Oceanogr. Lab. Rep. 1971-9: 10 p.

MCGOWAN, W. E., W. A. SANER, AND G. L. HUFFORD. 1974. Tar ball sampling in the western North Atlantic, p. 83–84. *In* Marine pollution monitoring. Proc. Symp. Workshop Natl. Bur. Stand., Gaithersburg, Md., May 13–17, 1974. Natl. Bur. Stand. Spec. Publ. 409.

MONAGHAN, P. H., D. E. BRANDON, R. A. BROWN, T. D. SEARL, AND J. J. ELLIOTT. 1974. Measurement and interpretation of nonvolatile hydrocarbons in the ocean. Part I. Measurements in Atlantic, Mediterranean, Gulf of Mexico, and Persian Gulf. U.S. Dep. Commer., Marit. Adm., Exxon Res. Eng. Co., Exxon Prod. Res. Co. AID.10 JB.74 EPR.4EX.74: 50 p.

MORRIS, B. F. 1971. Petroleum: tar quantities floating in the northwestern Atlantic taken with a quantitative neuston net. Science 173: 430–432.

MORRIS, B. F., AND J. N. BUTLER. 1973. Petroleum residues in the Sargasso Sea and on Bermuda beaches, p. 521–530. *In* Proc. Joint Conf. Prev. Control Oil Spills. Am. Pet. Inst., Washington, D.C.

SAMEOTO, D. D., AND L. O. JAROSZYNSKI. 1969. Otter surface sampler: a new neuston net. J. Fish. Res. Board Can. 25: 2240–2244.

Vertebral Number in North American Sculpins of the *Myoxocephalus quadricornis*-Complex

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MCALLISTER, D. E., AND J. ANISKOWICZ. 1976. Vertebral number in North American sculpins of the *Myoxocephalus quadricornis*-complex. J. Fish. Res. Board Can. 33: 2792–2799.

X-rays of 438 North American specimens of the *Myoxocephalus quadricornis*-complex (Cottidae) showed mean vertebral counts (including urostyle) of 38.433 in the deepwater sculpin which inhabits inland continental lakes; 41.097 in the freshwater relict in lakes on Victoria Island, Northwest Territories; 41.417 in newly discovered freshwater relicts in Nauyuk, Stanwell-Fletcher, and Sophia lakes, Northwest Territories; and 41.821 in the coastal fourhorn sculpin living in brackish and marine coastal waters and certain adjacent fresh waters. Except for the Victoria and the three lake relicts, the vertebral means of all pairs of these groups differed at $P < .01$ or $< .001$. Of the coastal fourhorn sculpin and the deepwater sculpin 91% are identifiable using only vertebral number, of the Victoria relict and the deepwater sculpin 68%, and of the Victoria relict and the coastal fourhorn only 10%.

Vertebral counts alone permit recognition of the deepwater sculpin as a subspecies, *Myoxocephalus quadricornis thompsonii* Girard, 1852, distinct from the arctic coastal fourhorn sculpin provisionally referred to *M. q. hexacornis* (Richardson, 1823) and suggest classification of the Victoria Island, Nauyuk, Stanwell–Fletcher, and Sophia lake relicts with the coastal form, rather than with the deepwater sculpin. Relicts in the latter three lakes are suggested to be postglacial in origin.

MCALLISTER, D. E., AND J. ANISKOWICZ. 1976. Vertebral number in North American sculpins of the *Myoxocephalus quadricornis*-complex. J. Fish. Res. Board Can. 33: 2792–2799.

Les radiographies de 438 spécimens nord-américains du complexe *Myoxocephalus quadricornis* (Cottidae) révèlent des comptages vertébraux moyens (urostyle compris) de 38.433 chez les chabots de profondeur qui habitent les lacs continentaux de l'intérieur; 41.097 chez les formes relictes d'eau douce des lacs de l'île Victoria, Territoires du Nord-Ouest; 41.417 chez les formes relictes d'eau douce nouvellement découvertes dans les lacs Nauyuk, Stanwell–Fletcher et Sophia, Territoires du Nord-Ouest; et 41.821 dans le chabot à quatre épines côtier habitant les eaux côtières saumâtres et marines, et certaines eaux douces avoisinantes. Sauf pour les formes relictes de l'île Victoria et des trois lacs, la moyenne vertébrale de toutes les paires de ces groupes diffère à des niveaux de $P < .01$ ou $< .001$. Chez le chabot à quatre épines côtier et le chabot de profondeur, 91% sont identifiables par le nombre vertébral seulement, 68% de la forme relicte de Victoria et du chabot de profondeur, et seulement 10% de la forme relicte de Victoria et du chabot à quatre épines côtier.

Les nombres vertébraux seuls permettent de reconnaître le chabot de profondeur comme sous-espèce, *Myoxocephalus quadricornis thompsonii* Girard, 1852, distincte du chabot à quatre épines côtier arctique connu provisoirement comme *M. q. hexacornis* (Richardson, 1823), et donnent à croire que les formes relictes des lacs de l'île Victoria et des lacs Nauyuk, Stanwell–Fletcher et Sophia devraient être classées avec la forme côtière plutôt qu'avec le chabot de profondeur. Les formes relictes de ces trois derniers lacs seraient d'origine postglaciaire.

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IN northern marine, brackish, and fresh waters of the Nearctic three distinctive allopatric populations of the *Myoxocephalus quadricornis*-complex are known. Their vernacular names and distribution are: 1) the fourhorn sculpin, a circumpolar coastal arctic form that sometimes ventures from its marine or brackish habitat into nearby rivers or lakes, and that is presumably the ancestral form. It may provisionally be referred to as *Myoxocephalus quadricornis hexacornis* (Richardson, 1823) on the assumption that it is distinct from the nominate form; 2) the deepwater sculpin, found in mainland lakes within or close to the basins of former glacial lakes of North America from the Great Lakes region and Ottawa River drainage west and north to Upper Waterton Lake, Alberta and Great Bear Lake, North-

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west Territories (Johnson 1975; Dadswell 1974; Scott and Crossman 1973; McAllister and Ward 1972); and 3) the Victoria relict, found in certain lakes on Victoria Island, N.W.T., in the Canadian arctic archipelago (Johnson 1964).

In the Palearctic three additional forms exist, the nominate brackish water Baltic fourhorn sculpin, *M. quadricornis quadricornis* (Linnaeus, 1758); the European relict found in Swedish, Finnish, and Soviet lakes adjacent to the Baltic (Segerstråle 1957) to which several subspecific names have been given; and the Krawchuk sculpin, *M. q. krawtschuki* Mikhalev, a viviparous form found in Lake Keta, USSR (Mikhalev 1973). Aside from the latter, the taxonomy of the Old World forms was treated by Berg and Popov (1932) and Berg (1949).

This paper contributes to understanding the taxonomy and origin of the North American fourhorn sculpin and its freshwater relicts as suggested by variation in vertebral numbers.

History

Before the discovery of the Victoria relict, the North American mainland deepwater sculpin, *M. thompsonii* Girard, 1852, had been considered specifically distinct from the coastal fourhorn sculpin, *M. quadricornis* (Linnaeus, 1758) by McAllister (1961) who also reviewed the earlier history of the problem. He found that all North American specimens of the two species could be separated by the graphical plot of preopercular spine length on standard length; frontal spines, parietal spines, cleithral spines, tubercles above and below the lateral line, and pectoral and anal fin ray counts showed significant but lesser differences.

Johnson (1964) reported the presence of *M. quadricornis* relicts in three lakes on Victoria Island, N.W.T., and forwarded the hypothesis that they were introduced by marine inundation following glaciation and then isolated in freshening lakes during subsequent uplift of the land. The evidence is not completely unequivocal. At about 10,000 BP the western face of the receding Keewatin ice sheet ran through Great Slave Lake, Great Bear Lake, and Victoria Island. Possibly melting ice provided a fresh water bridge to Victoria Island for Great Bear Lake populations. However, consideration of all evidence including the occurrence on Victoria Island of the relict isopod *Mesidothea entomon glacialis*, unknown in inland freshwaters of the mainland, leads us to concur with Johnson that the relicts were introduced from marine populations by inundation. McPhail and Lindsey (1970) studied the Victoria relict and found the preopercular spines to be intermediate in length between the fourhorn and deepwater sculpins, and the frontal, parietal, and cleithral spines to be better developed than in the deepwater sculpin. They stated that, "Probably the marine form has given rise to freshwater derivatives in several places and at several times: the process may well be continuing. Because we have no evidence that the two may occur sympatrically without interbreeding, and because the extreme forms seem joined by a continuum of morphological intermediates, we feel obliged to recognise only one species. On the other hand, the form inhabiting North American lakes seems to fulfill the criteria for subspecies described by Bailey et al. (1954), in that it displays a high degree of uniformity over a wide range and differs with high constancy from the marine form, with intermediates occurring only in a limited region." and concluded that, "The fish from lakes on Victoria Island . . . may be most conveniently considered as *M. q. thompsoni*, although this decision is on phenetic rather than phyletic grounds . . . [they] . . . probably evolved from marine ancestors more recently than did the southern deepwater fish."

Studies by Khan (1971) and Khan and Faber (1973) revealed that deepwater sculpin larvae lack the mediolateral row of melanophores found in fourhorn sculpin larvae, and have only 0–6 melanophores in the anisomeric medioventral row as opposed to 40–65 in fourhorn sculpin larvae (European relicts in Lake Vattern, Sweden, also lacked the mediolateral row but had about 20 medioventral melanophores). These differences between deepwater and fourhorn sculpins are nonoverlapping. The pigmentation differences, unlike those noted in adults by McAllister (1961), cannot be attributed to exposure of larvae to the feeble illumination of the depths since the larvae occur up to the surface. The number of myomeres in the larvae also differed; there were 37–42 myomeres in the deepwater sculpin and 42–45 in the fourhorn sculpin, with similar differences in vertebral numbers in a few adults examined. Vertebral count appeared to be a promising new character deserving investigation in a numerically and geographically adequate sample.

Materials and Methods

Four hundred and thirty-eight subadult specimens comprising 70 samples from 43 locations from the National Museum of Natural Sciences, Ottawa, the Royal Ontario Museum, Toronto, the University of Michigan Museum of Zoology, Ann Arbor, and the Smithsonian Institution, Washington, were x-rayed on Kodak type M2 Industrex X-ray film using a General Electric Mobile 90 II X-ray unit for large specimens, and a Super Soft X-ray Apparatus Softex model EMB unit for small specimens. Counts were made from the developed film using transmitted light. Urostylar vertebrae were included in the counts (see Fig. 1).

Geographical variation of vertebral means is indicated in Fig. 2. The Victoria Island relict sculpins were collected in or adjacent to areas inundated postglacially by the sea. All fourhorn sculpins were collected in the sea or within 20 miles of it, and all deepwater sculpins more than 100 miles from the sea in or close to former glacial lake basins. Subsamples of some major groups were drawn from similar latitudes to provide evidence on whether or

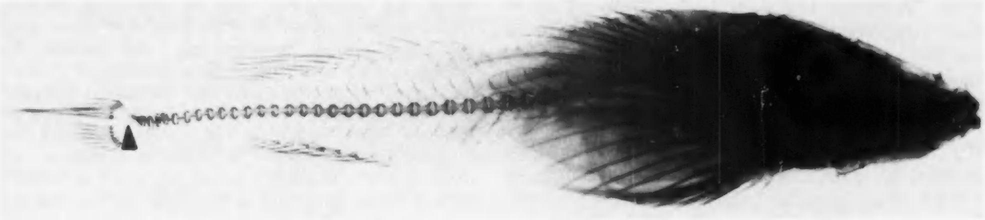

FIG. 1. X-rays of 128-mm specimen of deepwater sculpin from Lake Michigan, Mich., NMC64-719. Triangles indicate the first vertebra (atlas) and the last (urostyler) vertebra counted.

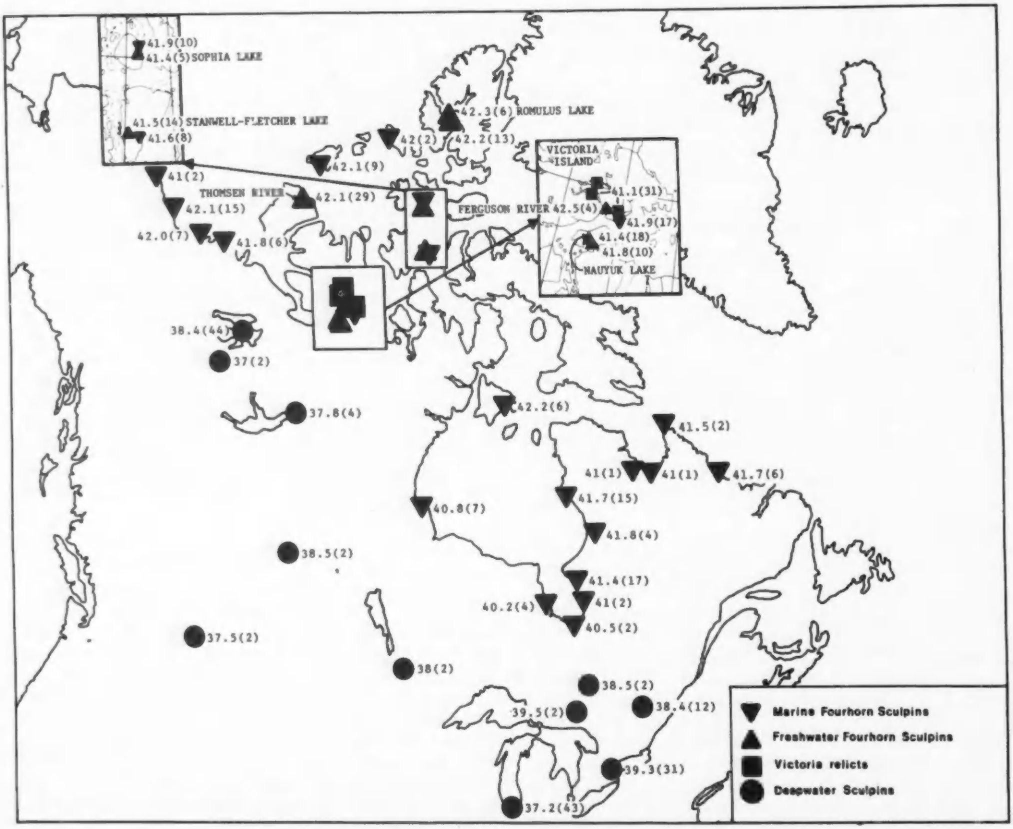
41.9(10)
41.4(5) SOPHIA LAKE
41.5(14) STANWELL-FLETCHER LAKE
41.6(8)
42.3(6) ROMULUS LAKE
42(2)
42.2(13)
42.1(9)
41(2)
THOMSEN RIVER
42.1(29)
42.1(15)
42.0(7)
41.8(6)
VICTORIA ISLAND
41.1(31)
FERGUSON RIVER 42.5(4)
41.9(17)
41.4(18)
41.8(10)
NAUYUK LAKE
38.4(44)
37(2)
37.8(4)
42.2(6)
41.5(2)
41(1)
41(1)
41.7(6)
41.7(15)
40.8(7)
41.8(4)
38.5(2)
41.4(17)
40.2(4)
41(2)
40.5(2)
37.5(2)
38(2)
38.5(2)
39.5(2)
38.4(12)
39.3(31)
37.2(43)
▼ Marine Fourhorn Sculpins
▲ Freshwater Fourhorn Sculpins
■ Victoria relicts
● Deepwater Sculpins

FIG. 2. Locations of samples studied (solid symbols) with vertebral means and, within parentheses, sample sizes. Samples within a lake or close together may be united and represented by a single value.

not the between-group differences are the result of latitudinal clines. Data were analyzed and graphed using a Hewlett-Packard 9830 minicomputer system and are summarized in Table 1 and Fig. 3.

When calculating the percentage identifiable using vertebral count, the number of specimens outside the zone of overlap of counts was multiplied by 100 and divided by the total number of each form separately. The two percentages were added together and divided by two to allow for disparities in sample size, to yield the percentage identifiable. T-tests were used to test whether the differences in means of the different groups were due to chance; $P < 0.01$ was considered statistically significant. A one factor analysis of vari-

TABLE 1. Statistics for, and differences between samples of vertebral numbers in the *Myoxocephalus quadricornis*-complex. F, fresh water; B, brackish water; S, salt water.

Sample no.	Form	Salinity	Area	No.	Range	$\bar{X}$	SD	C.V.
1)	Deepwater sculpin	F	Whole range	164	37–40	38.433	.934	2.431
2)	Deepwater sculpin	F	51°30′–66°20′ N	52	37–40	38.327	.923	2.408
3)	Victoria Island relict sculpin	F	Whole range	31	39–42	41.097	.790	1.922
4)	Fourhorn excluding three-lake samples	F, B, S	Whole range	207	40–43	41.821	.745	1.782
5)	Fourhorn sculpin	B, S	51°30′–66°20′ N	83	40–43	41.530	.786	1.893
6)	Fourhorn sculpin	F, B, S	60°05′–70°3′ N	36	40–43	41.944	.715	1.704
7)	Fourhorn sculpin	B, S	68°03′–79°55′ N	72	40–43	41.875	.649	1.549
8)	Fourhorn sculpin	B, S	Whole range	168	40–43	41.732	.754	1.807
9)	Three-lake fourhorns; Sophia, Nauyuk, & Stanwell–Fletcher lakes	F		36	40–43	41.417	.649	1.567
10)	Thomsen & Ferguson rivers & Romulus Lake	F		39	41–43	42.205	.570	1.351

ance of vertebral counts of the deepwater sculpin, the Victoria relicts, the three-lake relicts (see below), and nonrelict fourhorn sculpin yielded on F ratio of 550 with 3 and 434 df and a $P < 0.005$. Intergroup variation accounted for most of the variation (mean square of 367.648); the remaining variation was small (mean square of 0.668).

Results and Discussion

FRESHWATER AND BRACKISH-MARINE FOURHORNS

The mean vertebral count of the 39 Thomsen–Ferguson–Romulus specimens (42.205) is closest to but higher than that for the brackish–marine fourhorns from the same latitudes (Fig. 2). While a *t*-test shows that the difference in means for these two samples approaches significance ($P = 0.05–0.02$), only 1% of the specimens are correctly identifiable using vertebrae. As this percentage is not taxonomically significant, the two groups are united into a single fourhorn sample. Movement is possible between these populations and those in the sea. It is hypothesized that individuals moved upstream from river mouths during their life time and that this movement against the current favored larger individuals (which tend to have more vertebrae (Lindsey 1975)), since cruising and sprint speeds are positively related to body length (Webb 1975). Hubbs and Hubbs (1933) reported an analogous situation where the vigorous sunfish hybrids moved upstream against strong currents leaving the parental populations downstream. These freshwater fourhorns, according to our interpretation, do not constitute a gene pool distinct from that of the brackish–marine fourhorns.

Because there are only a few specimens from Nauyuk, Stanwell–Fletcher, and Sophia lakes and their means are very close, they are united for treatment into a single sample (the "three-lake sample") of 36 specimens with a mean of 41.417. They differ significantly from the united fourhorn sample ($P = 0.01–0.001$). The three-lake sample does not differ significantly ($P = 0.20–0.10$), from the Victoria Island relict sample of 31 specimens. But as the three-lake specimens reach 202 mm in total length (171 mm standard length) and the Victoria relicts (Johnson 1964) rarely exceed 70 mm (we have one 99 mm in total and 85 mm in standard length), the three-lake sample is kept separate. The three-lake sample is, however, highly different from the deepwater sculpins on the mainland ($P < 0.001$), and 90% of the two can be correctly identified using vertebral counts. We believe that the three-lake populations

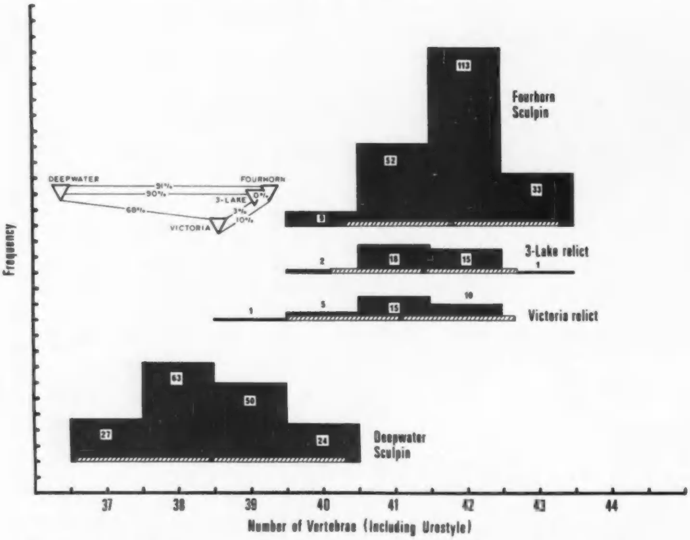

FIG. 3. Frequency of vertebral counts in the *Myoxocephalus quadricornis*-complex. At the base of each histogram the triangle indicates the mean, and the hatched rectangle shows two standard deviations on either side of the mean. The percentage of the pairs that can be correctly identified with vertebral counts is indicated in the inset.

constitute gene pools distinct from the brackish–marine population. Whether the size difference between them and the Victoria relicts is largely environmental or genetic is unknown at the moment. Possibly each of the arctic freshwater relicts arose separately, as the land, bearing freshening brackish lakes, rose isostatically. Southeastern Victoria Island was deglaciated about 10, Stanwell–Fletcher Lake 9.6, Nauyuk Lake 9.1, and Sophia Lake 9.0 thousand years BP (Geological Survey of Canada Map 1257A. Retreat of Wisconsin and recent ice in North America, 1969). Stanwell–Fletcher Lake remained directly connected to the sea until about 3500 BP and intermittently connected at flood tides until about 2400 BP according to Rust and Coakley (1970) who report on its limnology. Or it is possible that these three-lake populations arose in a common brackish glacial gulf or dispersed from one lake to another. The low coefficient of variation, 1.567, of the three-lake sample favors either of the latter alternatives.

The James Bay specimens had lower vertebral counts than most fourhorns. As vertebral counts frequently show clines with latitude (Lindsey 1975), a correlation coefficient between vertebrae and latitude was calculated for fourhorn sculpins yielding a coefficient of $r = 0.325$ (significant at $P < 0.001$). Examination of the means for 5° intervals of latitude from 50 to 80°N showed two steep increases then a leveling off: 41.174, 41.432, 42.043, 41.913, 42.026, 42.125, rather than an even slope.

James Bay specimens are not significantly different from Hudson Bay, Ungava Bay, and Labrador specimens to 60°N. The mean of the latter is significantly different at $P = 0.01–0.001$ from the more northerly specimens. None of the four 5° latitude samples from 60 to 80°N are significantly different from one another. So the coastal fourhorns divide into two groups, one south of 60° and one north of 60°N.

The sharp step between 60° and 65° and the rather constant mean counts of more northerly latitudes argues against the possibility that the shift is clinal, even though there is a small but significant correlation between vertebral count and latitude over the whole latitudinal range. Alternate explanations are 1) the injection of deepwater sculpin genes into Hudson and James bays via tributaries of Hudson Bay such as the Churchill River system, 2) the Wisconsin isolation of an eastern seabord population of fourhorns which subsequently invaded Hudson and James bays following the opening of this body of water about 8.2 thousand BP, 3) lower vertebral

TABLE 2. Pair comparisons; T value, % correctly identified, and probability that differences in vertebral means are due to chance. $^{*}P = 0.05–0.02$; $^{**}P = 0.01–0.001$; $^{***}P < 0.001$.

Sample	Form						
1	Deepwater (whole range)	1 Deepwater					
2	Deepwater (51 30′–66 20′)	—	2 Deepwater				
3	Victoria Isl. relict	14.25 68%***	—	3 Victoria			
4	Fourhorn, excl. 3-lake sample	35.61 91%***	—	4.34 10%***			
5	Fourhorn (51 30′–66 20′)	—	19.78 89%***	—			
6	Fourhorn (69 05′–70 03′)	—	—	3.99 11%***			
7	Fourhorn (68 03′–79 55′)	—	—	—	7 fourhorn		
8	Fourhorn (whole range)	—	—	—	—	8 fourhorn	
9	3-lake fourhorns	17.25 90%***	15.82 90%***	1.55 3%	2.78 0%**	3.14 0%**	9 3-lake
10	Thomsen & Ferguson rivers & Romulus L.	—	—	—	2.10 1%*	3.14 3%**	4.37 3%***

counts due to higher temperatures in these southern bodies of water. Evidence is insufficient to choose between these alternatives.

FOURHORN AND DEEPWATER SCULPINS

The united fourhorn sample was compared with deepwater sculpin drawn from its whole range in continental inland waters. The fourhorn sculpin sample had a mean vertebral count of 41.821, the deepwater sculpin sample a mean count of 38.433. The means show a highly significant difference ($P < 0.001$), and 91% of the specimens of the two forms are identifiable on the basis of vertebral counts. Because the fourhorn sculpins range farther to the north and deepwater sculpins farther to the south, we decided to exclude any differences associated with latitude. Sub-samples of both forms were drawn from between 51°30′ and 66°20′N, yielding significantly different ($P < 0.001$) vertebral means of 41.530 in fourhorn specimens and 38.327 in deepwater sculpin specimens; 89% of the specimens are correctly identifiable on the basis of vertebral counts. The difference between these two means for the same latitudes, 3.203, was nearly as great as that between the means for the whole range of each form, 3.388. The mean vertebral counts of deepwater sculpins from arctic and Atlantic drainages were not significantly different.

That 91% of deepwater and fourhorn sculpins drawn from their whole range can be correctly identified is worthy of subspecific recognition. To the vertebral distinction may be added the diagnostic features remarked by McAllister (1961); those noted by McPhail (1963) and the larval pigmentation characteristics reported by Khan and Faber (1973), may also prove to apply upon examination of a geographically more adequate sample. Additional study of these features may well warrant species status, but provisionally the deepwater sculpin can be recognised as a distinct subspecies, *Myoxocephalus quadricornis thompsonii* (Girard, 1852).

VICTORIA RELICTS AND DEEPWATER SCULPINS

The Victoria Island relicts have a mean vertebral count of 41.097. Deepwater sculpins do not reach as far north as the Victoria relicts but at the northern end of their range deepwater sculpins average 38.327 vertebrae. This, and the mean for specimens from over the whole range of the deepwater sculpins, 38.433, differs highly significantly ($P < 0.001$) from that of the Victoria relict; 68% of these two forms are correctly identifiable on the basis of vertebral number (Fig. 3). McPhail (1963) found differences between the Victoria relict and the deepwater sculpin in pyloric caeca, tubercles below the lateral line, and preopercular spine length.

VICTORIA RELICTS AND FOURHORN SCULPINS

Counts for the fourhorn sculpin from latitudes parallel to Victoria Island, 69°05′ to 70°03′N average 41.944 and are significantly different from the Victoria relicts ($P < 0.001$). Of these fourhorns and the Victoria relict sculpins only 11% are correctly identifiable on the basis of vertebral counts, while with fourhorn sculpins from over their whole range and the Victoria relicts 10% of the specimens are correctly identifiable. Percentage separations at these lower levels are not taxonomically significant.

The presently available morphological evidence on the relationships of the Victoria Island relict sculpin is equivocal; the pyloric caeca and vertebral counts of the Victoria relict are closer to the fourhorn, but the number of tubercles and the preopercular spine length as a percentage of the standard length are closer to the deepwater sculpin (McPhail 1963; present study). Vertebral evidence alone, because of only 10% separation, will not permit recognition of the Victoria relict as a subspecies distinct from the fourhorn sculpin. We, as well as Johnson (1964) and McPhail and Lindsey (1970) espouse the hypothesis that the Victoria relict arose independently and more recently from the fourhorn sculpin than did the deepwater sculpin. Contrary to McPhail and Lindsey, we believe in primacy of phylogenetic origin over phenetic resemblance, when they can be demonstrated to conflict, in relating and naming species-group taxa, i.e. independently derived forms, even if similar due to parallelism, should receive different names. Proceeding from that axiom, the Victoria relicts can be provisionally referred to as *M. quadricornis* (Linnaeus, 1758), while recognizing that, at a later date, they may well be shown to be subspecifically distinct from the coastal form. The North American coastal form cannot be referred at the moment to the nominate subspecies, *M. q. quadricornis*, because that name is based on the Baltic Sea relict whose status with respect to the arctic coastal form is uncertain. Berg and Popov (1932) believed the Baltic form to be subspecifically distinct from our marine form which he called *M. q. labradoricus* (Girard, 1850), but this is a synonym of *M. scorpius* (Linnaeus, 1758). The North American coastal form can be provisionally referred to as *M. q. hexacornis* (Richardson, 1823), the oldest available name.

In summary, Fig. 3 shows clearly the grouping of the three-lake relict and the Victoria relict with the fourhorn sculpin, rather than with the deepwater sculpin.

PARALLEL EVOLUTION

The North American freshwater forms have undergone parallel evolution; all have fewer vertebrae than their brackish–marine progenitor. Similarly, the Krawchuk sculpin (Mikhalev 1973) and the European lake relict (unpublished data) have fewer vertebrae than the arctic brackish–marine form. None of the freshwater relicts attains the size of the brackish–marine form.

According to Lindsey's Pleomeristic Rule (Lindsey 1975) fish populations or species of small maximum length in a given taxon tend to have fewer vertebrae than those of greater maximum length. The three-lake sample with a mean count of 41.417 reaches 171 mm in standard length, the Victoria relict with a mean of 41.097 vertebrae rarely exceeds 70 mm in length (Johnson 1964), the deepwater sculpin with a mean vertebral count of 38.422 reaches a maximum standard length of 199 mm, while the coastal fourhorn sculpin with a mean vertebral count of 41.821 attains a maximum standard length of about 280 mm (340 mm total length) in North America (Percy 1975). Vertebral counts and maximum body sizes of these populations are not strictly correlated, perhaps due to insufficient time for the younger populations to come into equilibria. However, all the freshwater relicts do have lower vertebral counts and smaller maximum lengths than the brackish–marine form. While the underlying basis for Lindsey's Pleomeristic Rule is unknown, the impinging selective pressure(s) must be strong, judging from the reduction in the number of vertebrae in all freshwater relicts of the *Myoxocephalus quadricornis*-complex, and in other fishes.

The coefficients of variation (see Table 1) are higher in the deepwater sculpin (2.431) and the Victoria Island relict sculpin (1.922), than in the fourhorn sculpin (1.774). There is probably little gene flow between most lake populations, whereas in the sea, gene flow is freer. Genetic differences are therefore permitted to accumulate in lakes but not in the sea. Alternately, lake temperatures may fluctuate more during incubation than sea temperatures and correspondingly influence vertebral variability.

It is noteworthy that all the postglacial freshwater relicts of *M. quadricornis* occur in regions lacking primary freshwater fishes. This and their euryhalinity has doubtless favored the evolution of these relicts.

Acknowledgments

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Berg, L. S. 1949. Freshwater fishes of U.S.S.R. and adjacent countries. Akad. Nauk U.S.S.R. Zool. Inst. 3: 929–1381.

Berg, L. S., and A. Popov. 1932. A review of the forms of *Myoxocephalus quadricornis* (L.). C. R. Acad. Sci. U.S.S.R. 6: 152–160.

Dadswell, M. J. 1974. Distribution, ecology and postglacial dispersal of certain crustaceans and fishes in eastern North America. Natl. Mus. Nat. Sci. (Ottawa) Publ. Zool. 11: 1–110.

Hubbs, C. L., and L. C. Hubbs. 1933. The increased growth, predominant maleness, and apparent infertility of hybrid sunfishes. Pap. Mich. Acad. Sci. Arts Lett. 17: 613–641.

Johnson, L. 1962. The relict fauna of Greiner Lake, Victoria Island, N.W.T., Canada. J. Fish. Res. Board Can. 19: 1105–1120.

1964. Marine-glacial relicts of the Canadian arctic islands. Syst. Zool. 13: 76–91.

1975. Distribution of fish species in Great Bear Lake, Northwest Territories, with reference to zooplankton, benthic invertebrates and environmental conditions. J. Fish. Res. Board Can. 32: 1989–2004.

Khan, N. Y. 1971. Comparative morphology and ecology of the pelagic larvae of nine Cottidae (Pisces) of the northwest Atlantic and St. Lawrence drainage. M.Sc. Thesis. Univ. Ottawa, Ottawa, Ont. 234 p. illus.

Khan, N. Y., and D. J. Faber. 1973. A comparison of the deepwater and fourhorn sculpin, *Myoxocephalus quadricornis* L. from North America. I. Morphological development, p. 703–712. *In* J. H. S. Blaxter [ed.] The early life history of fish. Springer-Verlag, Berlin, Heidelberg, and New York.

Lindsey, C. C. 1975. Pleomerism, the widespread tendency among related fish species for vertebral number to be correlated with maximum body length. J. Fish. Res. Board Can. 32: 2453–2469.

McAllister, D. E. 1961. The origin and status of the deepwater sculpin, *Myoxocephalus thompsonii*, a Nearctic glacial relict. Natl. Mus. Can. Bull. 172: 44–65.

McAllister, D. E., and J. C. Ward. 1972. The deepwater sculpin, *Myoxocephalus quadricornis thompsonii*, new to Alberta, Canada. J. Fish. Res. Board Can. 29: 344–345.

McPhail, J. D. 1963. The postglacial dispersal of freshwater fishes in northern North America. Ph.D. Thesis. McGill Univ., Montreal, Que. 167 p.

McPhail, J. D., and C. C. Lindsey. 1970. Freshwater fishes of northwestern Canada and Alaska. Bull. Fish. Res. Board Can. 173: 1–381.

Mikhalev, U. V. 1973. *Myoxocephalus quadricornis* (Linne) of Lake Keta (Pyasina Basin). Tr. Sib. Otd. Gos. Nauchno Issled. Inst. Ozern. Rechn. Rybn. Khoz. 8: 171–183. (Transl. from Russian in Natl. Mus. Nat. Sci., Ottawa, Ont.)

Percy, R. 1975. Fishes of the outer Mackenzie Delta. Dep. Environ., Beaufort Sea Proj., Victoria, B.C. 114 p.

Rust, B. R., and J. P. Coakley. 1970. Physico-chemical characteristics and postglacial desalination of Stanwell-Fletcher Lake, Arctic Canada. Can. J. Earth Sci. 7: 900–911.

Scott, W. B., and E. J. Crossman. 1973. Freshwater fishes of Canada. Bull. Fish. Res. Board Can. 184: 1–966.

Segerstråle, S. 1957. On the immigration of glacial relicts in Finland and adjacent Russian areas. Soc. Sci. Fenn. Commentat. Biol. 15: 1–36.

Webb, P. W. 1975. Hydrodynamics and energetics of fish propulsion. Bull. Fish. Res. Board Can. 190: 1–158.

Phosphorus Kinetics in Lake Water: Influence of Membrane Filter Pore Size and Low-Pressure Filtration

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LEAN, D. R. S. 1976. Phosphorus kinetics in lake water: influence of membrane filter pore size and low-pressure filtration. J. Fish. Res. Board Can. 33: 2800–2804.

Radiotracer kinetics using carrier-free ^{32}P-PO$_4$ were conducted on samples of water from Heart Lake, Ontario. Results obtained using 0.45-μm membrane filters were compared with those for 0.1 μm at vacuums of 400 mm Hg and to those for 0.45-μm filters using very low-pressure (4 mm Hg) filtration. The difference between 0.45 and 0.1 can reach 8–20% of the total radioactivity during the first 10 min of the experiment. After 60 min the fraction removed by 0.1, but not 0.45-μm filters, declines to only 1% of the total radioactivity, but this may represent as much as 50% of that which goes through 0.45 μm. The low-pressure filtration techniques provided similar results to those for normal filtration when kinetics were monophasic. Later in the season, the low-pressure method was shown to provide confusing artifacts that were explained by the hypothesis that tiny filaments extend from the surfaces of some species of aquatic algae and bacteria and are often dislodged during filtration.

LEAN, D. R. S. 1976. Phosphorus kinetics in lake water: influence of membrane filter pore size and low-pressure filtration. J. Fish. Res. Board Can. 33: 2800–2804.

Nous avons étudié la cinématique de traceurs radioactifs sur des échantillons d'eau du lac Heart, Ontario, en utilisant ^{32}p-PO$_4$ sans porteur. Nous avons comparé les résultats obtenus à l'aide de filtres à membrane de 0.45 μm avec ceux de membranes de 0.1 μm sous un vide de 400 mm Hg, et avec ceux de filtres de 0.45 μm sous très basse pression (4 mm Hg) de filtration. La différence entre 0.45 et 0.1 peut aller jusqu'à 8–20% de la radioactivité totale durant les 10 premières min de l'expérience. Après 60 min, la fraction qui est enlevée par les filtres de 0.1 mais non par ceux de 0.45 diminue à seulement 1% de la radioactivité totale, mais ceci peut représenter jusqu'à 50% de ce qui passe à travers 0.45 μm. La filtration sous basse pression donne des résultats semblables à ceux de la filtration normale, quand la cinématique est monophasée. Plus tard dans la saison, la méthode de basse pression produit des phénomènes secondaires qui prêtent à confusion et qu'on explique par l'hypothèse que de minuscules filaments sont présents à la surface de certaines expèces d'algues et de bactéries aquatiques, et que ces filaments sont souvent délogés au moment de la filtration.

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PHOSPHORUS radiotracer kinetics are usually conducted on lake waters using 0.45-μm membrane filters and vacuum filtration at 200–600 mm Hg to separate the particulate from the dissolved compartment (Riger 1973; Lean 1973a, b). In the following investigation, the kinetics obtained using these conventional conditions will be compared with those obtained using filters of smaller pore size and very low-pressure (4 mm Hg) filtration. There is good reason to suspect the existence of artifacts. Rigler (1973) found that 0.45-μm filters do not remove all the "particles" from lake waters and some of the "dissolved" P can be removed using 0.1-μm filters. These particles would appear as a part of the fraction termed colloidal P using gel-filtration techniques (Lean 1973a, b; Lean and Nalewajko 1976). In addition to particles not retained by filters, cells which are retained may become damaged. Many scientists have expressed concern that ^{14}C-labeled algae may release tracer because of cell damage during filtration (see Schindler et al. 1973). King (1970) argued that filtration caused a release of phosphate from the plankton.

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Materials and Methods

Samples of water from Heart Lake, a 17.6-ha eutrophic lake about 48 km northwest of Toronto, Ontario, were collected using a column sampler (Lean and Rigler 1974) and pooled to give a representative sample of the epilimnion. When returned to the laboratory, carrier-free ^{32}P-PO$_4$ (in dilute HCl from New England Nuclear or Atomic Energy of Canada) was diluted with sterilized distilled water, added to the lake water, and thoroughly mixed.

In the first part of the investigation, 5-ml samples were filtered through 25-mm, 0.45- and 0.1-μm Millipore membrane filters to remove the "particulate" fraction, at intervals until a constant distribution of ^{32}P between the filtrate and the particulate fraction was achieved. The vacuum was controlled to 400 mm Hg. At the same time, 200 ml of the sample of lake water plus the ^{32}P-PO_4 was added to a larger Millipore filter holder (for 47 mm diam filters) suspended over a series of test tubes. The height of the water above the filter created the hydraulic pressure needed for the gentle filtration. The depth of the 200 ml corresponded to only 4 mm Hg. When 1–4 ml of filtrate was collected, the entire assembly was moved to the next test tube.

The total radioactivity of the lake water and each of the filtrate samples was measured by pipetting 0.5 ml onto aluminum planchets. These were dried, covered with Parafilm to prevent accidental loss of isotope, and then counted in a Nuclear Chicago low background gas-flow counter.

The data were expressed as a percent ^{32}P in the filtrate and plotted on semilog graph paper as a function of time. The shapes of the curves derived using 0.45- and 0.1-μm filters at normal filtration vacuums and those using very low-pressure filtration were compared.

Since the uptake of ^{32}P-PO_4 is so rapid and the flow rate through the filters at low pressure is so slow, it is difficult to determine the time each sample represents. The arithmetic mean time should be used only for linear processes but the tracer kinetics are exponential. The greatest deviation would occur during the initial period. The general equation used to fit the uptake curve (Lean and Rigler 1974) can be simplified to

$$P_t = A\,e^{-kt}$$

for the first 5–10 min. P_t represents the percent isotope in the filtrate at any time (t), A is equal to the percent at the start of the time interval (Δx), and k is the unknown slope of the curve. The time (t) which halves the area under the curve for that sample is therefore

$$t = \frac{\ln[2/(e^{-k\Delta x} + 1)]}{k}.$$

The time data points were calculated using this relationship but were rarely < 0.4 of the time interval (Δx). In other words, they differed little from the arithmetic mean.

Results

TRACER KINETICS FOR 0.45- AND 0.1-μm FILTERS

The percent ^{32}P in the filtrate was plotted as a function of time after addition of ^{32}P-PO_4 to the Heart Lake water (Fig. 1). These curves represent periods where the ^{32}P-PO_4 was rapidly taken up by the particulate forms. The initial slope (divided by 2.303) approximates the rate constant for phosphate uptake. The phosphate turnover time (time required for an amount of phosphate to be taken up equivalent to that present) is the reciprocal. Typical phosphate turnover times were 2–3 min with the exception of June 26, 1969 when the turnover time was only 0.9 min. The difference between the percent through 0.45 and 0.1 μm is also shown. Generally, the maximum difference occurred during the first 10 min of the experiment, after which the differences were small (see July 9, September 5, and June 11). The kinetics obtained using both 0.45 and 0.1 μm are similar on June 18, 1968 and the filtration times for 0.1 μm are short compared with all other dates. On June 26, 1969, the time for filtration using 0.1-μm filters was so long that these data were discarded. Only the 0.45-μm data are included here to illustrate the extremely rapid flux for phosphate.

Clearly, in the first three examples in Fig. 1, there appear to be some small particles that are quickly labeled with ^{32}P-PO_4 that pass the

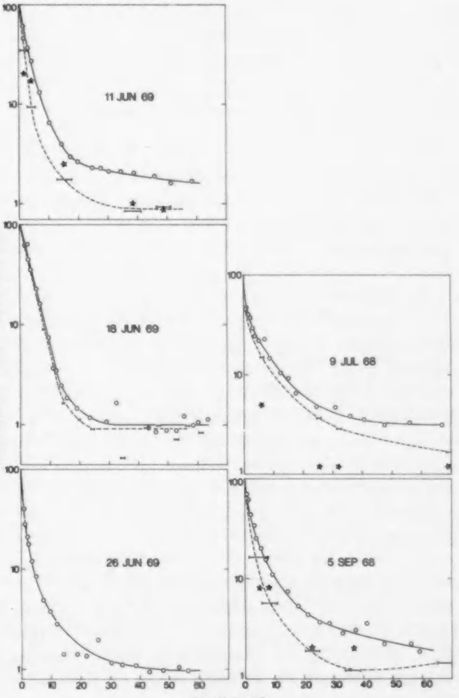

FIG. 1. Graphs of percent ^{32}P in the filtrate as a function of time for 0.45-μm (—○—) and 0.1-μm (|————|) filters. Also shown as (★) is the difference between the filters. The horizontal lines denote the filtration time for 0.1 μm filtration.

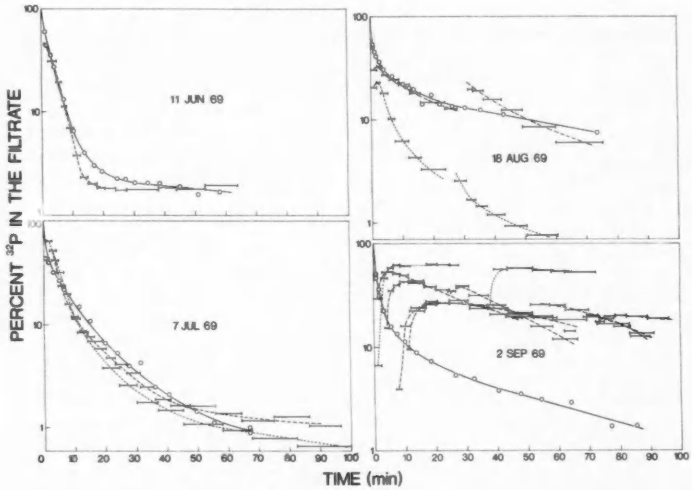

FIG. 2. Graphs of percent ^{32}P in the filtrate as a function of time for 0.45-μm (—○—) filters at normal filtration vacuums (————), and at low-pressure filtration (|————|). The horizontal line denotes the time for collection of the sample. The long dash line represents the curve using 0.45 μm at low pressure filtration, and the short dash line for 0.22 μm also using the low pressure method.

0.45-μm filter but are retained by a 0.1-μm filter. Later, over the next 60 min, these forms decline to only 1–2% of the total activity. They could sufficiently alter the shape of the 0.45-μm uptake curve and contribute to the disphasic shapes discussed earlier (Lean 1973a, b). The kinetics for June 18, 1969 are consistent with those for a two-compartment exchange model (monophasic) and no significant differences between the two filters were found. Apparently, a form of phosphorus that can represent 8–20% of the total activity (during the early part of a kinetic experiment) exists when filtration times for 5 ml through 0.1-μm filters exceed about 4 min. At first glance this would seem to have little to do with algae since most would also be collected on 0.45 μm. The filtration times for 5 ml through 0.45 μm were consistently < 30 s. As one might expect, 0.1-μm filters removed about half of the fraction termed colloidal P by gel-filtration analyses (Lean 1973c). Although the differences between 0.45 and 0.1 μm are greatest during the first 10 min of the experiment, after 60 min the 0.1-μm filters often remove 50% of that fraction not removed by 0.45.

Experiments using 0.1-μm filters were attempted during July–August but with the exception of July 9, 1968, filter plugging problems were so severe and filtration times so long that the data were unreliable.

Further evidence that some small "particle" contributes to long filtration times was obtained during the experiments on ^{32}P-PO$_4$ abiotic complexing (Lean and Rigler 1974). During 1966–69 Heart Lake water was filtered through 47 mm diam, 0.45-μm filters. When monophasic curves were obtained large (400 ml) volumes could be filtered and the flow rate of the filtrate through ion-exchange columns was similar to that for distilled water. Later, when extreme diphasic kinetics were observed, only 50–70 ml could be filtered and the flow rate of filtered lake water through the ion-exchange columns required vacuum to maintain rates similar to those for distilled water. Both observations are consistent with the hypothesis that something in the 0.45-μm filtrate causes the water to be more viscous.

TRACER KINETICS USING LOW-PRESSURE FILTRATION

Results from June 3, 11, 18, 26, and 30, 1969, obtained with the low-pressure filtration method, were similar to those for normal vacuum filtration. As the month progressed, the uptake became more rapid. Turnover times for phosphate shortened from 9 min for June 3 to < 1 min on June 30. The June 11 data are given in Fig. 2 as an example typical of this period. The kinetics were usually consistent with those for a two-compartment exchange system.

The low-pressure filtration method was developed using 0.45-μm filters but 0.22-μm filters could also be substituted. Experiments were conducted on July 7, 10, 16, and 23. Since the results differed little from each other, only the July 7 data are given (Fig. 2). Differences in the first 5 min were observed but otherwise neither the filters nor the filtering technique resulted in significantly different curves. This, unfortunately, did not last.

Experiments were conducted on August 3, 5, and 18 with the latter shown on Fig. 2. There was a considerable difference between the 0.22- and 0.45-μm low-pressure methods with more activity removed by 0.22; the filters tended to plug up quickly and the volumes collected for each sample were less than volumes collected in June or July. At the same time, the differences in the results obtained using normal vacuum (0.45) and the low-pressure filtration (0.45) methods occurred only in the initial 5 min. The first sample often fell below the rest of the curve.

Filter plugging problems and poor replication were so severe in early September that it was necessary to run several replicates with regular filter changes. The 0.45 μm obtained using normal vacuum filtration seemed like most diphasic uptake curves but the low-pressure filtration curves were totally unexpected and existing theory could not explain the results. Instead of the 2% asymptote, the level was at least 20% for 0.45 and over 60% for the 0.22 filtration at low pressures. In addition, the initial points were always below the rest of the curve. ^{32}P-PO$_4$ added to filtered lake water and then treated as above for whole lake water showed that this could be partly due to retention of ^{32}P-PO$_4$ by the filter, a phenomenon not detected earlier in the season. Blank values, using the vacuum filtration method, never exceeded 2%.

After recording the results of the September 2, 1969 filtration, we concluded that the normal vacuum filtration method probably creates fewer artifacts than that for low pressure.

Discussion

Differences between the data obtained using 0.45- and 0.1-μm fiilters (at normal vacuums) were greatest in the period from 0 to 10 min after ^{32}P-PO$_4$ was added to lake water. This may result from artifacts caused by stripping tiny filaments from the algae and bacteria in lake water as the ^{32}P moved from the filtrate to the filterable fractions.

Recently, Paerl and Lean (1976 this issue) and G. G. Leppard et al. (unpublished data) have demonstrated the presence of various kinds of fine filaments extending from algae. Cagle (1974) has made similar discoveries for bacteria. These filaments were found only as a result of new refinements in microscopic technique. Individual fibrils are only 0.002–0.020 μm diam and appear as long delicate ribbons. They are fragile and probably eventually slough off into the natural environment. In the laboratory such fibrils definitely are partly dislodged during filtration. Their importance to this discussion is profound. When phosphate is taken up, it would not be unreasonable to find it is taken up directly by the filaments. If they are stripped off during filtration, they would appear as colloidal P or the fraction not retained by 0.45-μm filters but partly retained by 0.1-μm filters.

During the kinetic experiments presented by Lean (1973c), the colloidal P was labeled quickly and then declined. The same was shown here for the fraction removed between 0.1 and 0.45 μm.

The volume collected by the low-pressure filtration method decreased as the season progressed. As the algae settled on the surface of the filters, their filaments would tend to follow the currents into the pores of the filter. If they were broken off, they would appear in the filtrate and the percent ^{32}P-filaments to filtrate volume would be less for 0.45 filters at normal vacuum than for 0.45 filters using low-pressure filtration and even less for 0.22 at low-pressure filtration. This may possibly explain the September 2, 1969 data.

Evidence that cells are damaged during filtration is still essentially lacking but it now seems obvious that filaments are stripped off (G. G. Leppard et al. unpublished data). King's (1970) observation that an amount of orthophosphate was released equivalent to that present can also be explained by Lean's (1973b) Sephadex gel-filtration studies where the distribution of the isotope between the phosphate and nonphosphate forms was measured immediately after filtration and then at intervals up to 5.5 h thereafter. The peak for phosphate often doubled during this period. In other words, King's observation can be explained without postulating cell damage. With this in mind, there is no compelling evidence to merit switching to any low-pressure filtration method. In fact, some surprising artifacts may be produced.

Certain complicating events happen simultaneously. Significant differences in the kinetics obtained using 0.45- and 0.1-μm filters at normal vacuums exist when kinetics are diphasic. This occurs when the colloidal P (by gel-filtration analyses) was shown to contribute to the diphasic kinetic experiments. During these periods long filtration times and filter plugging problems are encountered; also, the kinetics obtained at low pressures differ from those obtained using a vacuum of 400 mm Hg.

Summary and Conclusions

As algae deplete phosphorus in lake waters the turnover time of phosphate becomes more rapid. This seems to be followed by a period when the tracer kinetics are disphasic and when it is more difficult to filter the lake water using 0.1-μm filters. Attempts to minimize cell damage by using reduced pressure filtration can often cause other surprising artifacts. It is during these periods when the role of colloidal phosphorus has been previously identified (Lean 1973a, b). The potential significance of tiny filaments projecting from the algae was hypothesized but more work is required to fully appreciate their significance. It may be that colloidal P is produced simply by stripping off filaments during filtration.

CAGLE, G. D. 1974. Fine structure and distribution of extracellular polymer surrounding selected aerobic bacteria. Can. J. Microbiol. 21: 395–408.

KING, P. H. 1970. A test of the hypothesis that vacuum filtration of lakewater releases orthophosphate. M.Sc. Thesis. Univ. Toronto, Toronto, Ont. 87 p.

LEAN, D. R. S. 1973a. Phosphorus dynamics in lakewater. Science 179: 678–680.

1973b. Phosphorus movement between biologically important forms in lake water. J. Fish. Res. Board Can. 30: 1525–1536.

1973c. Phosphorus compartments in lakewater. Ph.D. Thesis. Univ. Toronto, Toronto, Ont. 199 p.

LEAN, D. R. S., AND C. NALEWAJKO. 1976. Phosphate exchange and organic phosphorus excretion by freshwater algae. J. Fish. Res. Board Can. 33: 1312–1323.

LEAN, D. R. S., AND F. H. RIGLER. 1974. A test of the hypothesis that abiotic phosphate complexing influences phosphorus kinetics in lakewater. Limnol. Oceanogr. 19: 784–788.

PAERL, H. W., AND D. R. S. LEAN. 1976. Visual observations of phosphorus movement between algae, bacteria, and biotic particles in lake water. J. Fish. Res. Board Can. 33: 2805–2813.

RIGLER, F. H. 1973. A dynamic view of phosphorus in lakes, p. 539–572. *In* E. Griffith et al. [ed.] Environmental phosphorus handbook. John Wiley and Sons, Inc., Toronto, Ont.

SCHINDLER, D. W., R. V. SCHMIDT, AND R. A. REID. 1972. Acidification and bubbling as an alternative in determining phytoplankton production by the ^{14}C method. J. Fish. Res. Board Can. 29: 1627–1631.

Visual Observations of Phosphorus Movement Between Algae, Bacteria, and Abiotic Particles in Lake Waters

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The transfer of ^{33}P-PO$_4$ into plankton and the appearance of labeled colloidal material occurs within minutes in lake water. It is difficult to clearly separate the microscopic forms involved in phosphorus transfer by mechanical means since size ranges of algae, bacteria, detritus, and colloidal matter overlap. To circumvent this problem we used microautoradiography and scanning electron microscopy (SEM) to describe the sequence in which bacteria, algae, and suspended particles remove ^{33}P-PO$_4$ from solution and the physical appearance of excreted material suspected of containing labeled phosphorus.

Bacteria initially showed much more dense labeling with ^{33}P-PO$_4$ but, since the algal biomass greatly exceeded the bacterial biomass, algal ^{33}P-PO$_4$ incorporation represented the most significant uptake pathway. After 2 h ^{33}P accumulated in both the detrital aggregates which were harboring bacteria and the "capsular" material surrounding *Anabaena*. When these suspected extracellular release sites were examined by SEM, we observed fibrillar and amorphous particles of colloidal size (.05–0.5 μm). The possible ecological roles of these colloidal substances are discussed.

PAERL, H. W., AND D. R. S. LEAN. 1976. Visual observations of phosphorus movement between algae, bacteria, and abiotic particles in lake waters. J. Fish. Res. Board Can. 33: 2805–2813.

Le transfert de ^{33}p-PO$_4$ dans le plancton et l'apparition de matériel colloïdal marqué se produisent en dedans de quelques minutes dans de l'eau de lac. Il est difficile de séparer clairement, par des moyens mécaniques, les formes microscopiques impliquées dans le transfert du phosphore, étant donné qu'il y a chevauchement des gammes de taille des algues, des bactéries, des détritus et de la matière colloïdale. Afin de contourner cette difficulté, nous avons utilisé la microautoradiographie et la microscopie électronique à balayage (MEB) pour décrire l'ordre dans lequel les bactéries, les algues et les particules en suspension enlèvent le ^{33}p-PO$_4$ de la solution et l'apparence physique du matériel excrété soupçonné de contenir du phosphore marqué.

Les bactéries montrent au début un marquage beaucoup plus dense au ^{33}p-PO$_4$. Par contre, étant donné que la biomasse algale dépasse de beaucoup la biomasse bactérienne, l'incorporation du ^{33}p-PO$_4$ par les algues représente la voie d'assimilation la plus importante. Au bout de 2 h, le ^{33}p-PO$_4$ s'accumule à la fois dans les masses de détritus abritant des bactéries et dans le matériel « capsulaire » entourant *Anabaena*. En examinant au MEB les sites soupçonnés de dégagement extracellulaire, nous observons des particules fibrillaires et amorphes de taille colloïdale (.05–0.5 μm). Nous discutons des rôles écologiques possibles de ces substances colloïdales.

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RAPID transfer of radioactive phosphorus from dissolved phosphate to the particulate fraction has been observed in lake waters during summer (Rigler 1956, 1973; Lean 1973a, b) but the relative importance of uptake by algae, bacteria, or abiotic particles remains largely conjectural. Using autoradiography combined with tracer kinetics, we showed that the "uptake" was initially by bacteria and phytoplankton. Other sites of radiotracer accumulation were identified but these were below the limit of sensitivity of the light microscope. Scanning Electron Microscopy (SEM) revealed that these "particles" may represent the

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colloidal forms discussed by Lean (1973a, b) and Lean and Nalewajko (1976).

Much of the earlier work in this area is open to criticism. For example, Rigler (1956) showed that the fraction < 5 μm was responsible for the rapid uptake of ^{32}P-PO$_4$ in Toussant Lake and that the uptake was stopped by using antibiotics (tetramycin and penicillin) or heat. Later (1973) he criticized the use of antibiotics for two reasons. First, his discovery that the phosphate concentration was much lower than indicated by chemical analysis suggested that even a small loss of phosphate by the treated cells would apparently block the uptake. Second, the effect of antimetabolites may not be restricted to bacteria. He did, however, make a contribution that changed the generally accepted idea of the time. It had been thought that algae take up nutrients during photosynethesis and bacteria regenerate nutrients through decomposition. Rigler showed that bacteria grown in lake water do take up phosphate. Burnison (1974) using a combination of size fractionation and isotope labeling (^{32}P-PO$_4$, ^{14}C-HCO$_3$, ^{14}C-glucose) showed that in the eutrophic Bay of Quinte the initial uptake of phosphorus was by algae and bacteria.

Other researchers have attempted to use Michaelis-Menton (M-M) constants to indicate the affinity of algae vs. bacteria for phosphate (Rhee 1972, 1973; Fuhs 1972). However M-M techniques imply a net uptake of nutrient, whereas, in contradiction to the conclusions reached in Fuhs' review (1972), bacterial and algal cells exchange phosphorus with the medium (Lean and Nalewajko 1976; D. R. S. Lean unpublished data). Also, M-M techniques involve adding substrate levels of 100–1000 times typical levels of biologically available P to natural lake waters. Thus, the approach used by Wright and Hobbie (1965) in separating algal from bacterial uptake mechanisms by the use of M-M plots may not clearly illustrate separate uptake mechanisms under natural conditions in a diverse planktonic community.

Materials and Methods

Lake water was collected from Heart Lake, a 17-ha eutrophic lake situated in a sedimentary watershed 48 km northwest of Toronto, Ontario. This lake, at the time of our experiment, July 14, 1975, had a chlorophyll *a* concentration of 40 μg/liter. The total, soluble unreactive, and soluble reactive phosphorus concentrations were 70, 10, and < 2 μg P/liter, respectively. Composite samples (0–2 m) were collected and immediately taken to the laboratory at Burlington, Ontario.

To conduct kinetic experiments in parallel with autoradiographic examination the soft β emitter ^{33}P was preferred over ^{32}P. Transfer rates using ^{33}P-PO$_4$ were conducted as described by Lean and Rigler (1974). Four μCi of ^{33}P-PO$_4$ were added to about 400 ml of lake water and aliquots of 5 ml withdrawn and filtered at times as shown in Fig. 1. A control was prepared by adding a similar amount of ^{33}P-PO$_4$ to a second flask containing 400 ml of a 0.5% solution of formaldehyde in filtered lake water.

The 0.45-μm Millipore filtrate ^{33}P forms were char-

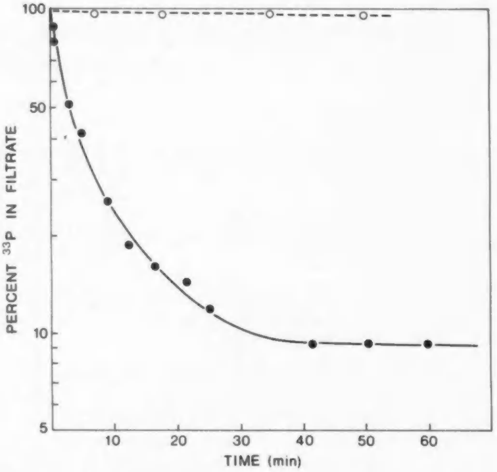


FIG. 1. Uptake of ^{33}P-PO$_4$ with time, July 14, 1974, for lake water (—●—) and lake water plus formaldehyde (—○—).

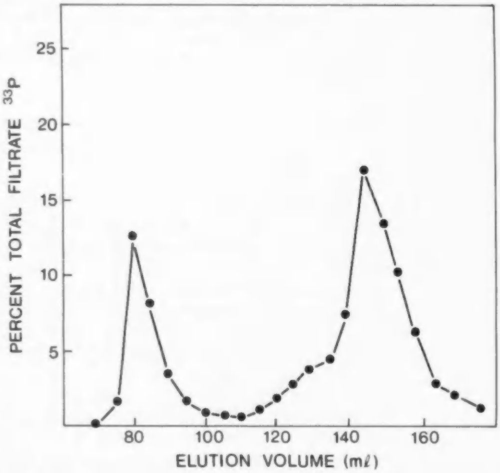


FIG. 2. Sephadex fractions in Heart Lake filtrate after 1 h. The three peaks contain 30, 12, and 58% (left to right). The peak on the left has been termed colloidal P, the small second peak is XP, while the third is phosphate.

acterized after 60 min using G-25 Sephadex (Pharmacia, Sweden) gel filtration as described by Lean (1973a, b). Samples (2.5 ml) were injected and 0.2% NaCl and 0.02% sodium azide used as the eluant.

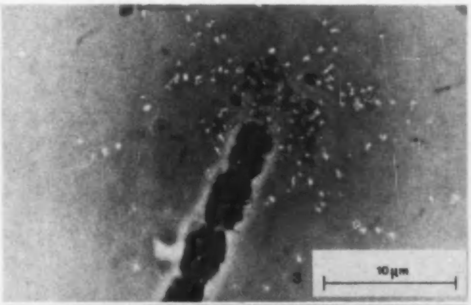
Following initial ^{33}P inoculation, subsamples were withdrawn from untreated and formaldehyde-treated flasks at 2-, 10-, 25-, 60-, and 120-min intervals. Autoradiographs were made of 0.5-ml vol filtered at 200 torr on 0.2 μm porosity Sartorius filters (25 mm diam). Filters were stained with 5% Erythrosin-B, destained with filtered lake water, and optically cleared by fuming them in acetone while attached to microscope slides (Paerl and Goldman 1972; Paerl 1974). Cleared filters were then immersed in Kodak NTB-2 Nuclear track emulsion which had been heated to 45 C and exposed for periods from 3 to 7 wk at 0 C (Paerl 1974). Exposures were developed for 2 min in Agfa Rodinal, diluted 50:1, and observed by oil-immersion phase microscopy at 1000 ×.

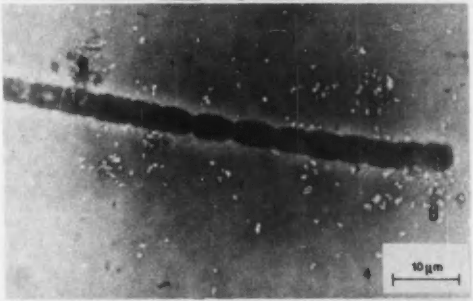
Samples for scanning electron microscopy (SEM) were taken at the start of the experiment. Both 0.5- and 1.0-ml of whole lake water as well as the 0.45-μm (Sartorius filters) filtrate were filtered (200 torr) on 0.2-μm porosity Nuclepore filters (25 mm). Preparation of SEM samples and observations were made according to methods described by Paerl and Shimp (1973).

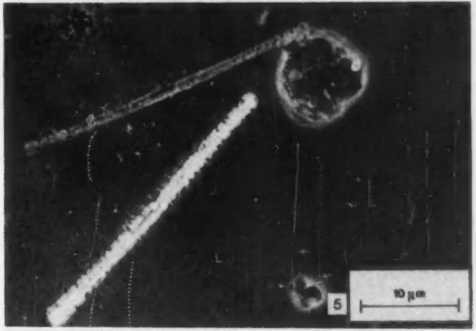
Results and Discussion

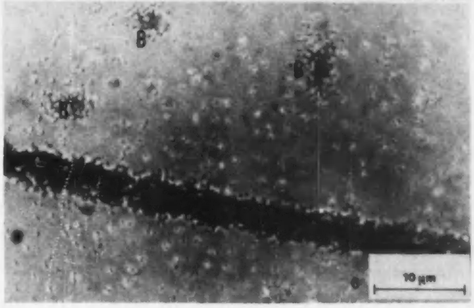
The uptake of ^{33}P-PO$_4$ (Fig. 1) was very similar to that obtained for ^{32}P-PO$_4$ during July for Heart Lake, 1967–70 (Lean 1973c). The initial slope (converted to ln scale) approximates the rate constant for phosphate uptake and is equal to 0.2 min^{-1}. The reciprocal is equal to the turnover time for phosphate (5 min). The sample containing formaldehyde showed no significant uptake.

When samples were removed from the experimental flask for autoradiographic analysis, an additional 25 ml was also fixed with formaldehyde to stop the uptake of ^{33}P-PO$_4$. At 12.3 min, 21% of the ^{33}P-PO$_4$ was in the filtrate and after an additional 3, 24, 27, and 43 min the percents were 25, 21, 22, and 25, respectively. This shows that the formaldehyde prevented further uptake and, equally important, did not cause ^{33}P to leak









FIGURES 3–6. Autoradiographic observations on the sequence of ^{33}PO$_4$ labeling.

FIG. 3. ^{33}P uptake after 2 min of incubation. Bacterial uptake preceded algal uptake as shown in this photograph. Exposed silver grains are white as opposed to black to enhance contrast of underlying microbes. FIG. 4. ^{33}P uptake after 10 min of incubation. Bacteria (B) appear heavily labeled and in a majority of cases the underlying cells are completely covered by silver grains. *Anabaena* is beginning to show ^{33}P uptake. FIG. 5. ^{33}P uptake after 25 min of incubation. Bacterial (B) ^{33}P uptake compared to ^{33}P uptake by diatoms in Heart Lake water. The exposed silver grains are black in this photograph to enhance the contrasting diatom frustules. FIG. 6. ^{33}P uptake after 2 h of incubation. *Anabaena* is now completely covered by silver grains as are filtered bacteria (B) near the filament.

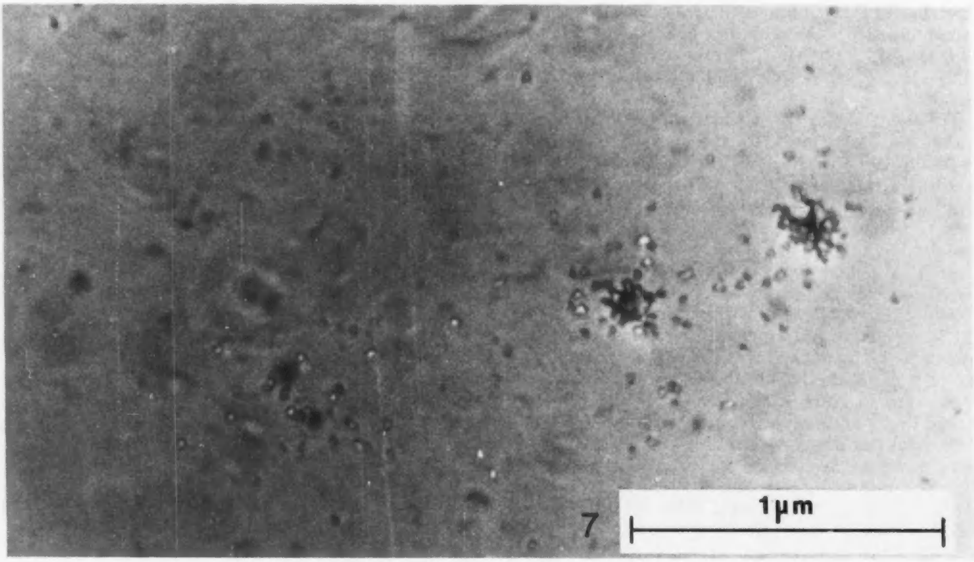


FIG. 7. Hot spots present on filters (0.45 µm) after a 2-min incubation of Heart Lake water with ^{33}P-PO$_4$. No apparent microorganisms were present underneath these spots. Similar spots showed up in refiltered filtrate (0.45 µm) samples. (Silver grains are black in this exposure).

from the particulate fraction. Similarly, at 60 min no changes in the percent ^{33}P in the filtrate took place after the uptake was blocked with formaldehyde. Even after 15 h, the percent rose from 9.2 to only 15.

The retention or refiltration of the 60-min filtrate was measured by filtering 5 ml lake water through a 0.45-µm Millipore filter, then refiltering immediately. A 0.45-µm filter retained 14% of the filtrate, whereas 0.22-µm filter retained 25%. The percent of the filtrate was almost double that of when Reeve Angel ultrafilters were used. Upon refiltration, 80% of this could removed using an 0.22-µm Millipore. Retention on refiltration is most pronounced when the "colloidal P" is labeled.

Sephadex gel filtration analysis of the filtrate obtained 60 min after ^{33}P-PO$_4$ was added to the lake water is shown in Fig. 2. The filtrate contained 9.2% of the total radioactivity. Gel filtration divided this into three fractions: colloidal P, an unidentified intermediate form (XP), and PO$_4$, containing 30, 12 and 58%, respectively. The XP form appears as a shoulder just before the PO$_4$ peak. The void volume for G-25 represents those molecules of molecular size exceeding 5000. Previous work (Lean 1973a, b) showed that the apparent molecular size exceeded 10^6.

The uptake of ^{33}P-PO$_4$ was followed by autoradiography. (Space does not permit a complete sequence of observations to be given and autoradiogram photos do not provide as much information as obtained by the investigator using the microscope.) Nevertheless, the autoradiographic examinations showed that after 2 min of ^{33}P exposure, bacteria had become labeled (Fig. 3). Phytoplankton labeling could be observed after 10 min but the specific activity (in terms of silver grain counts) per unit biomass was approximately 30% of that for bacterial cells (Fig. 4). The specific activity in algal cells gradually increased after 10 min (Fig. 5) but never attained levels observed in bacteria-sized particles. However, since algal biomass exceeded bacterial biomass by a considerable degree, total algal ^{33}P incorporation exceeded bacterial ^{33}P incorporation. The *Anabaena* showed gradual labeling over 2 h, whereas that for bacteria was much more rapid.

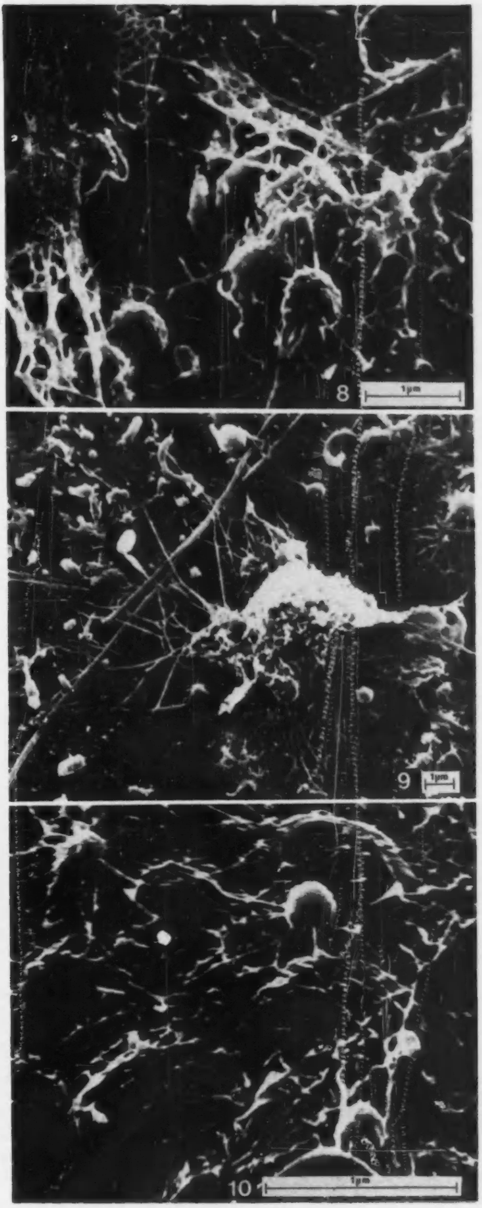
Some detrital aggregates supported a microflora that had actively incorporated ^{33}P. After 24 h, ^{33}P labeling of detritus was distributed throughout the particles, showing a general movement spreading away from attached bacterial cells. These findings parallel the earlier observation on ^{3}H-organics initially incorporated by bacteria, followed by movement throughout detrital particles as an excreted product of metabolism (Paerl 1974).

Formaldehyde-treated samples failed to show incorporation of ^{33}P in particulate matter, algae, and bacteria, when liquid scintillation and autoradiographic examinations were made. This treatment would have identified any false sources of exposure on autoradiographs but no sources were seen.

Dense spots of radioactivity, not overlying any apparent particle, were noticed on filters as early as 2 min after ^{33}P-PO$_4$ inoculation of water samples (Fig. 7). Similar hot spots were noted by Fuhs and Canelli (1970), Stull (1971), and Paerl and Perkins (unpublished data). No visible microorganisms were found underneath. These spots may represent colloidal P, discussed earlier by Lean (1973a, b, 1976) and Lean and Nalewajko (1976), which has been retained by the membrane filter. Alternatively, they could signify actively metabolizing microbes of a size range smaller than can be resolved with the light microscope.

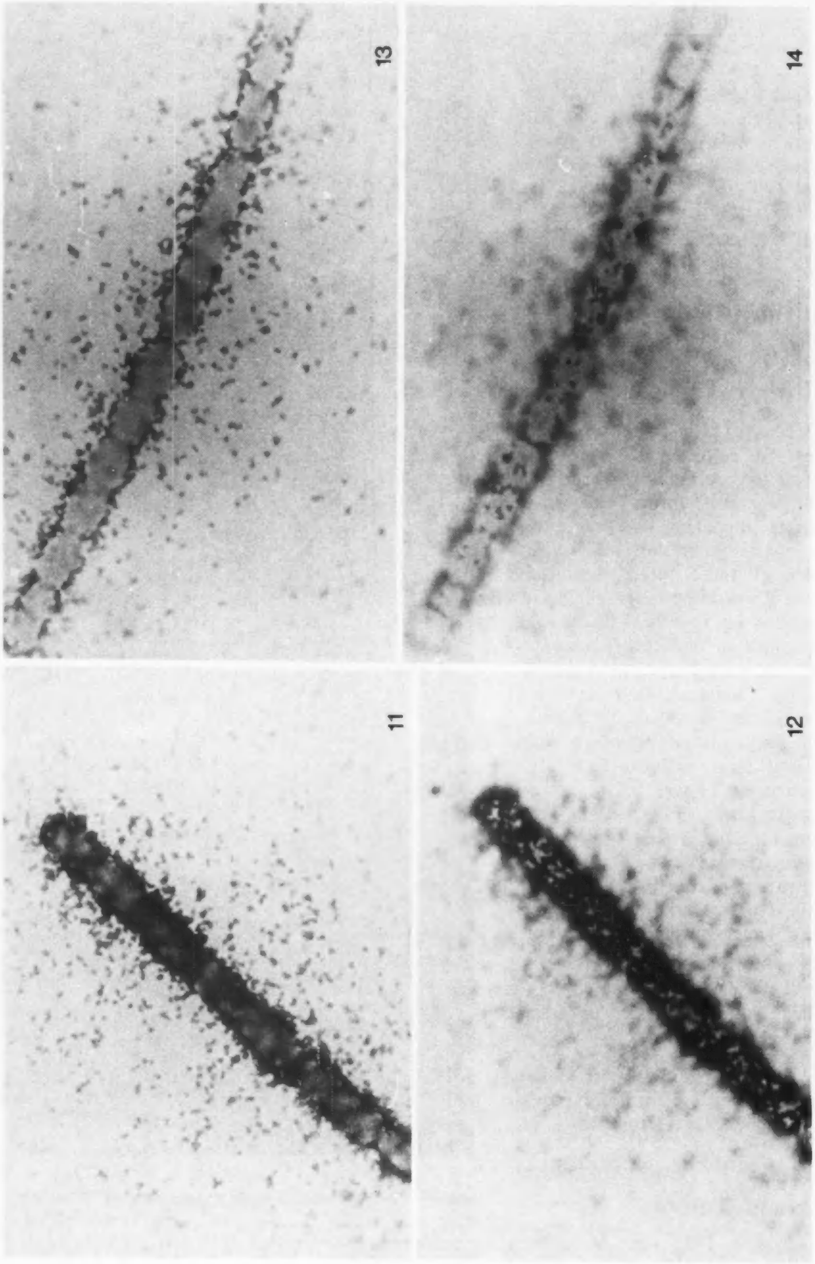
Two lines of evidence suggest that hot spots, at least in part, represent colloidal P. Firstly autoradiographic examination of the 0.45-μm filtrate refiltered on 0.45-μm filters shows diminished, yet similar, hot spots appearing on the second set of filters. Thus, assuming similar size-fractionation characteristics of these filters, additional retention of colloidal P would be more likely than refiltration of microbes by identical filters. Secondly, SEM observations of particulates down to 0.05-μm size reveal few particles resembling bacteria in that size-class. However, abundant small (.05–0.5 μm) fibrous and amorphous particles resembling excreted polymers commonly encountered in bacterial and algal cultures and larger flake-like detrital particles (Paerl 1974) can be seen on the Nuclepore filters (Fig. 8–10). These small particles appear to be broken or sloughed off from the surface of microbes. The material retained upon refiltration of the 0.45-μm filtrate is shown in Fig. 10. This material is not derived from Millipore or Nuclepore filters themselves since distilled water blanks show no such structures. Such particles are not resolved by light microscopy. These may be the same as the "hot spots" observed in Fig. 7, i.e. the colloidal ^{33}P fraction.

The formation of extracellular ^{33}P by the larger phytoplankters was observed on autoradiographs. Whole lake water samples from Heart Lake and



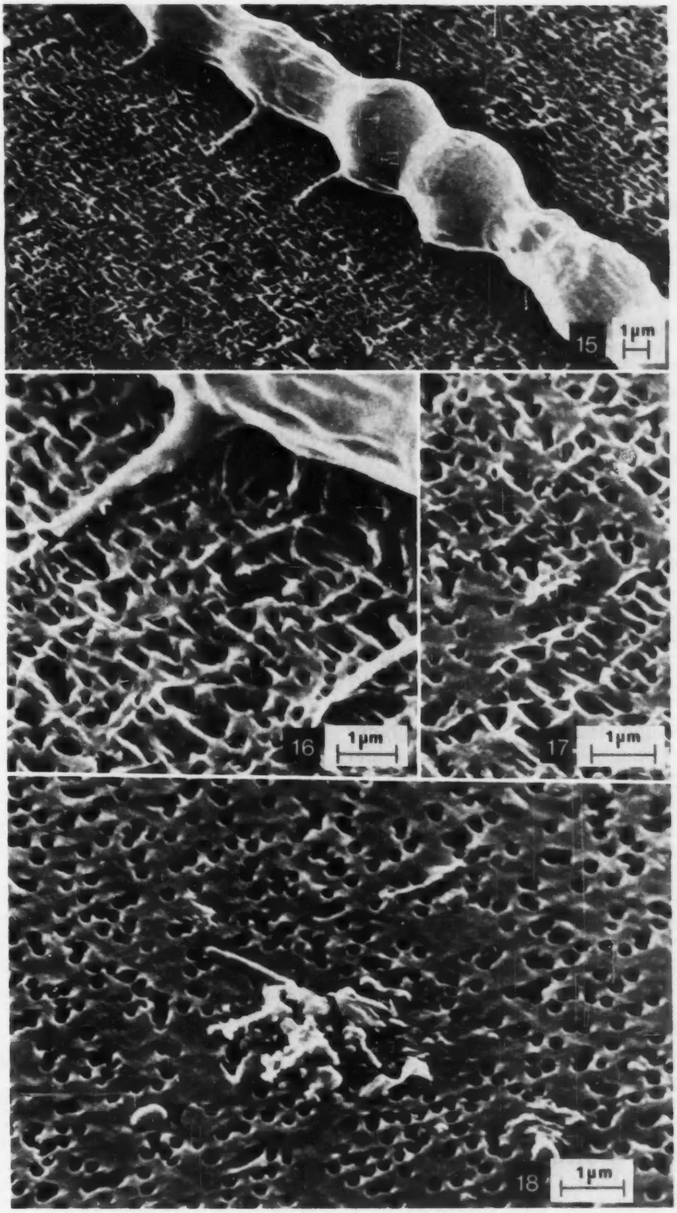
FIGURES 8–10.

FIG. 8. High magnification SEM view of bacteria embedded in a network of excreted material. Fibrils seen here are colloidal in size and also appear on filters supporting refiltered filtrate. FIG. 9. Flake-like aggregate of bacteria plus excreted fibrillar matrix in filtered Heart Lake sample. Pore size of Nuclepore filter used is 0.2 μm. Note the extent of extracellular material associated with free-floating bacteria as well. FIG. 10. Ultrahigh magnification SEM shows material retained upon refiltrations. Small bacteria-like particles (0.15–0.2 μm) seem to have filaments extending from them linked to other particles.



FIGURES 11–14. Autoradiographs of the blue-green *Anabaena* from Heart Lake.

FIG. 11. After a 4-h incubation period with ^{33}P-PO$_4$. FIG. 12. Change in focus on the autoradiograph shown in Fig. 11 to demonstrate the silver-grain exposure directly over the same filament. FIG. 13. ^{33}P labeled *Anabaena* filament resuspended in ^{33}P free-lake water for 20 h. These filaments were still capable of photosynthetic $^{14}CO_2$ uptake and thus were considered viable. Note silver grains extend from the cell wall and may be associated with sections of the filament that are not detected by light microscopy. FIG. 14. Change in focus on the autoradiograph shown in 13. Most of the ^{33}P is no longer detected directly over the filament but appears near the cell wall.



FIGURES 15–18.

FIG. 15. *Anabaena* filament settled by gravity filtration (to avoid damage to the cells) on to a 0.2-μm porosity Nuclepore filter. Note the major trails of extracellular material leading onto the filter. FIG. 16. High magnification photograph showing coverage of the pores in the Nuclepore filter near the same *Anabaena* filament. The excreted product covering these pores appears to be mucoid-like, represented by stacks of fibrils laid down on the filter surface. Width of the filament is approximately 3.5 μm. FIG. 17. High magnification photograph showing the reappearance of pores in the Nuclepore filter due to decreasing coverage by excreted products of *Anabaena*. The field shown is approximately 15 μm distant from the filament of Fig. 16. FIG. 18. Pores in the Nuclepore filter completely free of coverage by excreted products of *Anabaena*. The field shown is approximately 30 μm distant from the filament of Fig. 17 and 16. The clump of material shown was unidentified.

Lake Rotongaio, New Zealand (both lakes have *Anabaena* as a dominant phytoplankter) were inoculated with trace concentrations of ^{33}P-PO$_4$. Movement of the tracer throughout the *Anabaena* chain was monitored during a 24-h incubation period. After an initial 4-h period, which should allow for thorough incorporation of the tracer, the cells were washed by gentle filtration of 5-μm porosity Nuclepore filters, followed by resuspension in ^{33}P-free lake water (Fig. 11 and 12). In both lakes, the label moved from within the cell to capsular material outside the cell wall, without apparent cellular damage or lysis. Resuspended cells showed photosynthetic uptake of ^{14}C-CO$_2$, thus remaining viable after filtration and resuspension. After 20 h, small bits of detached labeled material accumulated in the medium near filtered specimens and most of the ^{33}P still associated with *Anabaena* was found in the capsule (Fig. 13 and 14).

SEM observations of *Anabaena* from both lakes revealed extensive extracellular material, some of which resembled the colloidal particles discussed earlier. After glutaraldehyde fixation, the capsular material is of mixed fibrillar and amorphous form. It extends at least several filament diameters away from the cell walls, completely covering the distinctive round pores in the Nuclepore filter (compare pores, Fig. 15–17 vs. 18). These observations reveal extracellular material surrounding *Anabaena*. Autoradiographs indicate that this material harbors ^{33}P after initial uptake by *Anabaena* (Fig. 13 and 14). Small detached clumps of this material can be seen dispersed on Nuclepore filters near the main sheath of capsular material. If such detachment is common in nature, as Walsby (1968) has indicated in studies on mucous excretion by gliding *Anabaena* filaments, it could represent a source of colloidal ^{33}P detected by Sephadex filtration.

Conclusions

We suggest that constituents of a colloidal pool of phosphorus are microscopically similar to extracellular products of cell metabolism. The existence of such colloidal fragments appears to be widespread. Similar observations have been made on a variety of lakes ranging in trophic state from oligotrophic (Lake Tahoe, California) and mesotrophic (Lake Michigan; Lake Taupo, New Zealand) to eutrophic (Devils Lake, North Dakota, Clear Lake, California). The question remains, why do phytoplankton and bacteria excrete copious amounts of phosphorus when reliable evidence exists that phosphorus is a growth-limiting factor in most lakes? At present, diverse hypotheses can be generated regarding the ecological significance of findings presented here. Our concluding remarks seemingly contain only a few of the potentially plausible explanations.

On inspection of biotic extracellular materials with SEM, much of the mucoid colloidal material is found near microbial cells, whether it be in detrital aggregates, flakes, or on single cells. It may be advantageous from an energetic point of view for microbial cells (especially those cells not possessing polyphosphate bodies) to deposit and store new sources of inorganic phosphorus in bound slime layers and capsular material. The cell would thereby place itself near a potential source of phosphorus in a phosphorus-poor environment where enzymatic accumulation of dissolved orthophosphate may be extremely difficult. Some extracellular phosphorus appears to be sloughed off from the surfaces of cells and detritus. Although lost from the surface, such organic substances may be broken down (by the use of extracellular enzymes) more readily than phosphorus adsorbed and precipitated by inorganic matter including clays, silts and large particulates (Stotzky 1970). In effect, the excretion of colloidal phosphorus (or excretion of a compound of low molecular weight which is then joined to a colloid) may be a means of maintaining phosphorus availability in aquatic ecosystems facing rapid losses to the sediments by adsorption to sinking precipitates and particles or in deep-water pelagic systems (i.e. North Pacific gyre–Sargasso Sea) where regeneration by vertical mixing rarely occurs.

Apparently, colloidal matter must be reutilised and transformed by microorganisms. If the organic fraction present in the 0.45-μm filtrate is largely made up of macromolecules, colloidal concentrations would build up to enormous proportions (given suspended high rates of extracellular formation) without conversion to particulate matter through detritus formation or decomposition.

Acknowledgments

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BURNISON, B. K. 1974. Microbial ATP studies. Verh. Int. Ver. Limnol. 19: 286–290.

FUHS, G. W. 1972. Microbial influences on phosphorus cycling p. 149–169. *In* L. J. Guarraia and R. K. Ballentine [ed.]. The aquatic environment: The microbial transformations and water management implications. EPA 430/G-73-008.

Fuhs, G. W., and E. Canelli. 1970. Phosphorus-33 autoradiography used to measure phosphate uptake by individual algae. Limnol. Oceanogr. 15: 962–966.

Lean, D. R. S. 1973a. Phosphorus dynamics in lakewater. Science 179: 678–680.

1973b. Phosphorus movement between biologically important forms in lake water. J. Fish. Res. Board Can. 30: 1525–1536.

1973c. Phosphorus compartments in lakewater. Ph.D. Thesis. Univ. Toronto, Toronto, Ont. 199 p.

1976. Phosphorus kinetics in lake water: the influence of membrane filter pore size and low pressure filtration. J. Fish. Res. Board Can. 33: 2800–2804.

Lean, D. R. S., and C. Nalewajko. 1976. Phosphate exchange and organic phosphorus excretion by freshwater algae. J. Fish. Res. Board Can. 33: 1312–1323.

Lean, D. R. S., and F. H. Rigler. 1974. A test of the hypothesis that abiotic phosphate complexing influences phosphorus kinetics in lakewater. Limnol. Oceanogr. 19: 784–788.

Paerl, H. W. 1974. Bacterial uptake of dissolved organic matter in relation to detrital aggregation in marine and freshwater systems. Limnol. Oceanogr. 19: 966–972.

Paerl, H. W., and C. R. Goldman. 1972. Heterotrophic assays in the detection of water masses at Lake Tahoe, California. Limnol. Oceanogr. 17: 145–148.

Paerl, H. W., and S. L. Shimp. 1973. Preparation of filtered plankton and detritus for study with scanning electron microscopy. Limnol. Oceanogr. 18: 802–805.

Rhee, G. Y. 1972. Competition between an alga and an aquatic bacterium for phosphate. Limnol. Oceanogr. 17: 505–514.

1973. A continuous culture study of phosphate uptake, growth rate and polyphosphate in *Scenedesmus* sp. J. Phycol. 9: 495–506.

Rigler, F. H. 1956. A tracer study of the phosphorus cycle in lake water. Ecology 37: 550–562.

1973. A dynamic view of phosphorus in lakes, p. 539–572. *In* E. Griffith et al. [ed.] Environmental phosphorus handbook. John Wiley and Sons, Inc., Toronto, Ont.

Stotzky, G. 1970. Clay minerals and microbial ecology. Trans. N.Y. Acad. Sci. 30: 11–21.

Stull, E. A. 1971. Primary productivity of individual phytoplankton species in Castle Lake, California. Ph.D. Thesis. Univ. California, Davis, Calif. 151 p.

Walsby, A. E. 1968. Mucilage secretion and the movements of blue-green algae. Protoplasma 65: 233–238.

Wright, R. T., and J. E. Hobbie. 1965. The uptake of organic solutes by plankton, bacteria, and algae. J. Ocean Sci. Ocean Eng. 1: 116–127.

Notholca laurentiae and *N. michiganensis*, New Rotifers from the Laurentian Great Lakes Region

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STEMBERGER, R. S. 1976. *Notholca laurentiae* and *N. michiganensis*, new rotifers from the Laurentian Great Lakes region. J. Fish. Res. Board Can. 33: 2814–2818.

The descriptions of two new planktonic rotifers, both cold stenotherms, is presented. *Notholca laurentiae* n.sp. is a predominant winter rotifer of the Laurentian Great Lakes, but to date has not been found in inland lakes of the region. Morphologically its closest relative is the European brackish water species, *N. salina* Focke.

Notholca michiganensis n.sp. has been recorded only in the oligotrophic inland lakes of the northern portion of Michigan's lower peninsula during winter and early spring (water temperatures 0.5–5.2 C). Morphologically, *N. michiganensis* is similar to, but distinctly smaller than *N. frigida* Jaschnov.

STEMBERGER, R. S. 1976. *Notholca laurentiae* and *N. michiganensis*, new rotifers from the Laurentian Great Lakes region. J. Fish. Res. Board Can. 33: 2814–2818.

Nous décrivons deux rotifères planctoniques nouveaux, tous deux des sténothermes d'eau froide. *Notholca laurentiae* n.sp. est un rotifère prédominant en hiver dans les Grands lacs laurentiens, mais il n'a pas jusqu'à maintenant été trouvé dans les lacs intérieurs de la région. Morphologiquement, son plus proche parent est l'espèce d'eau saumâtre européenne, *N. salina* Focke.

Notholca michiganensis n.sp. a été signalé seulement dans les lacs oligotrophes de l'intérieur de la partie septentrionale de la péninsule inférieure du Michigan en hiver et au début du printemps (températures de l'eau de 0.5–5.2 C). Morphologiquement, *N. michiganensis* est semblable à *N. frigida* Jaschnov, mais nettement plus petit.

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THE genus *Notholca* Gosse, 1896 (Rotifera: Brachionidae) is an important component of the zooplankton in the Great Lakes region. Members of the genus are most prevalent during winter months, but species such as *N. labis* Gosse, *N. acuminata* (Ehrbg.), and *N. acuminata* var. *extensa* (Olofsson) may also occur in warmer water temperatures. The primary cold-water species are *N. foliacea* (Ehrbg.), *N. squamula* (Müller), and the two new species described herein. These four species often form the most important constituents of the winter limnetic rotifer fauna in the upper Great Lakes region.

Materials and Methods

Zooplankton samples were collected quarterly for a 2.5-yr period (1973–75) with a no. 20 nylon mesh (76 μ) 0.25-m diam plankton net in 40 inland lakes in northern lower Michigan. Great Lakes samples were collected at 78 stations in southern Lake Huron and Saginaw Bay (April–November 1974) during eight separate cruises, with 8-liter Niskin bottles. In northern Lake Michigan, samples were collected at 55 stations during spring 1976 with 5-liter Niskin bottles. All samples were relaxed with carbonated water and preserved with 5% formalin. Zooplankton samples were also provided by the U.S. Fish and Wildlife Service from the water intake at the Hammond Bay field station near Rogers City, Michigan. Zooplankton samples collected bimonthly (1972–73) with a 9-liter Van Dorn bottle in Lake Michigan off Milwaukee were also included. Lake Erie zooplankton samples for spring 1976 were provided by the Center for Lake Erie Area Research, Ohio State University. Over 1600 zooplankton samples were analyzed, which encompassed all seasons of the year for both upper Great Lakes and inland lakes of Michigan.

A 1-ml subsample from concentrated samples containing the specimens was placed into a Sedgewick–Rafter counting cell. Morphological measurements were then made with a compound microscope at 100 or 200×. The holotypes were pipetted onto a glass slide and mounted with a cover glass in polyvinyl lactophenol containing lignin pink dye.

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Notholca laurentiae n.sp.

HOLOTYPE

National Museum of Natural Sciences, Ottawa, NMC 3803. Single female (mounted on a glass slide) collected in Lake Huron, 44° 10′ N, 82° 05′ W at a depth of 20 m, June 5, 1974.

PARATYPES

NMC 3804. 12 females taken from the same sample as the holotype, preserved with 5% buffered formalin (in vial).

DESCRIPTION

Females: anterior margin of dorsal plate with 6 spines; anterior margin of ventral plate has 6 folds. Dorsal surface of lorica strongly striated (Fig. 1A–F). Body corpulent in lateral view, with a distinct cleft in posterior margin between dorsal and ventral plates (Fig. 2). Mean total lorica length 225.5 μ; mean lorica width 142 μ at its widest point. Median dorsal spines (25 μ mean length) recurve ventrally; intermediate dorsal spines (9.9 μ) longer than lateral dorsal spines (5.4 μ). In dorsal view posterior margin of dorsal plate rounded, although frequently gently rounded bulge in posterior extremity of ventral plate exists (Fig. 2).

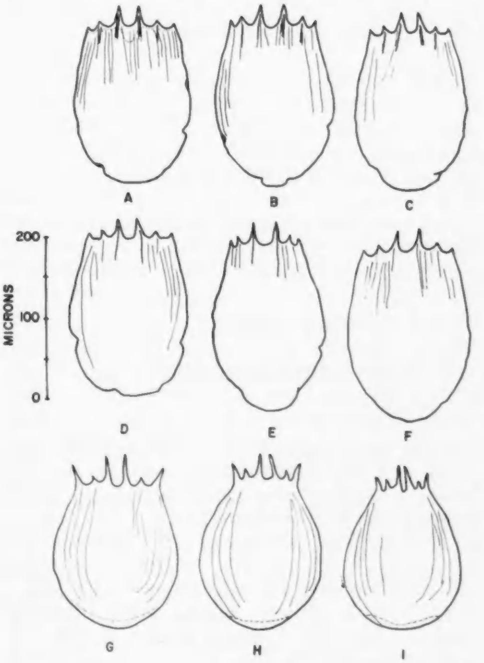

FIG. 1. Typical variation of lorica shape (dorsal view) of *Notholca laurentiae* (A–F) and *N. michiganensis* (G–I).

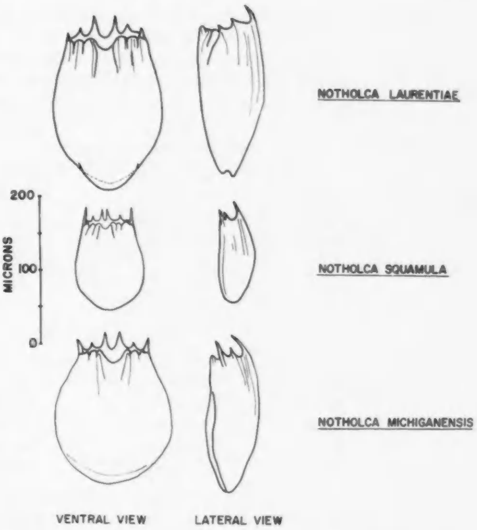

FIG. 2. Ventral and lateral views of 3 species of *Notholca*.

Males of *N. laurentiae* have not been observed.

DISCUSSION

Notholca laurentiae is morphologically similar to, but distinctly larger than the salt water species *N. salina* Focke, known from the coastal waters of Europe. *Notholca laurentiae* has a mean total lorica length of 226 μ as compared to 143 μ (Björklund 1972) for *N. salina*. In Lakes Michigan and Huron no individuals exceeded a total length of 250 μ. However, Nauwerck (1972) recorded *N. striata* (= *laurentiae*) from Lake Ontario ranging in total length from 200–285 μ.

The median and intermediate dorsal spines are longest in *N. laurentiae* and *N. salina*, whereas the dorsal median and lateral spines are longest in *N. squamula* (and *N. michiganensis* n.sp.) (Table 1). In lateral view, the body is corpulent in *N. laurentiae* and flattened in *N. squamula* (and *N. michiganensis* n.sp.).

Variations in body shape, including indentations or wrinkles on the lorica as well as the presence of a short, rounded posterior extension, may be due in part to the degree of contraction of the lorica after fixation with formalin (Fig.

TABLE 1. Descriptive measurements (in microns) for three species of *Notholca*. Abbreviations: TLL = total length of lorica; LW = lorica width (body width); MDS = median dorsal spines; IDS = intermediate dorsal spines; LDS = lateral dorsal spines; W/L = lorica width/total lorica length.

	Range	Mean	Mean error	SD	*N*
		Notholca laurentiae			
TLL	192–250	225.54	0.46	10.37	232
LW	115–163	142.08	0.32	7.15	232
MDS	14–33	24.96	0.20	4.60	232
IDS	6–15	9.94	0.16	1.75	53
LDS	4–8	5.38	0.13	1.21	37
W/L	0.49–0.86	0.63	0.002	0.04	232
		Notholca squamula			
TLL	112–160	133.43	0.41	9.17	230
LW	70–109	90.19	0.35	7.88	228
MDS	12–25	19.15	0.12	2.31	169
IDS	7–16	9.53	0.22	1.78	30
LDS	10–16	14.03	0.11	1.44	40
W/L	0.55–0.79	0.68	0.002	0.05	228
		Notholca michiganensis			
TLL	180–220	199.51	0.31	7.64	269
LW	110–180	144.85	0.30	7.29	269
MDS	18–38	28.51	0.20	4.15	205
IDS	5–18	9.58	0.10	1.82	153
LDS	15–25	19.67	0.27	2.22	30
W/L	0.54–0.88	0.73	0.001	0.04	269

1A–F). Björklund (1972) noted similar alterations of body shape in *N. salina*. The general morphology of the lorica places *N. laurentiae* in the *N. squamula* group of closely related species.

Historically, investigators of Great Lakes rotifers have erroneously considered *N. laurentiae* and *N. squamula* as a single species mistakenly called *N. striata* (Müller) (Forbes 1883; Jenning 1896; Ahlstrom 1934, 1936; Chandler 1940; Davis 1954, 1962). Nauwerck (1972) noted discrepancies between Müller's *N. striata*, found in the brackish waters of the European coast, and the species found in the Great Lakes, but considered the Great Lakes form identical to *N. squamula frigida* Jaschnov (= *N. frigida*). Nauwerck (1972) did, however, clearly distinguish *N. striata* (= *laurentiae*) from *N. squamula*. Stemberger (1974) noted that *N. striata* of the Great Lakes was sufficiently different from the European species to be considered a new species, based on the fact that the Great Lakes taxon does not possess movable lateral spines and that it is known to occur only in fresh water.

Notholca laurentiae occurred from late October through mid-July in Lake Michigan (Stemberger 1974), but was present throughout the year at deepwater stations in Lake Huron (unpublished data). The species occurred at water temperatures ranging from 0.5 to 18.0 C, and reached its peak abundances between 6 and 9 C. During thermal stratification, *N. laurentiae* is present in small numbers in the hypolimnion.

Notholca laurentiae, a cold stenotherm, is apparently restricted to the Laurentian Great Lakes as it has not been recorded in any of the deep water inland lakes of the northern portion of Michigan's lower peninsula. If *N. salina* were found in North American coastal waters, support would be given for recognizing *N. laurentiae* as a possible glaciomarine species.

Notholca michiganensis n.sp.

HOLOTYPE

NMC 3801. Single female (mounted on a glass slide) collected in Burt Lake, 45° 28′ N, 84° 40′ W, Cheboygan County, Michigan, USA, March 5, 1975.

PARATYPES

NMC 3802. Fifteen females taken from the same sample as the holotype, preserved with 5% buffered formalin (in vial).

DESRIPTION

Females: anterior margin of dorsal plate with

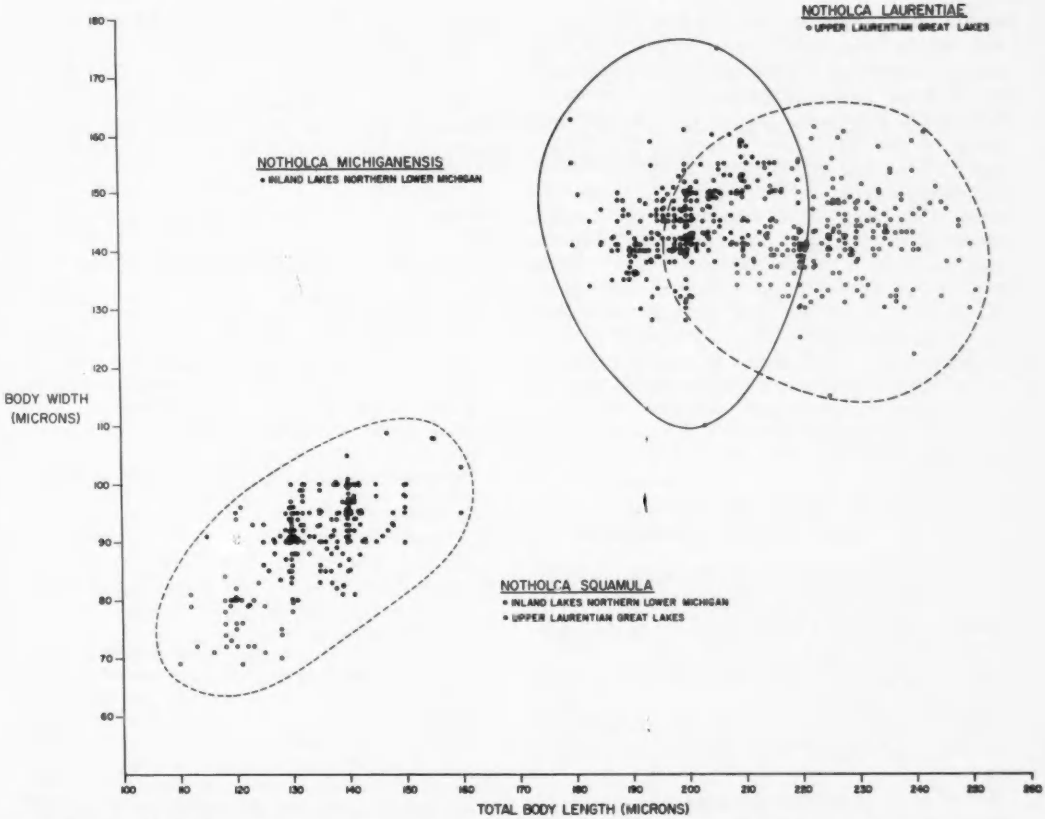

FIG. 3. Variation between total body length (total lorica length) and body width (lorica width) of *Notholca* spp. Broken lines enclose population variation of *N. squamula* and *N. laurentiae*, and solid line encloses variation in *N. michiganensis*.

6 spines, anterior margin of ventral plate has 6 folds. Dorsal surface of lorica distinctly striated (Fig. 1G–I); body somewhat compressed in lateral view, continuous and rounded in the posterior extremity (Fig. 2). Well-rounded dorsal and ventral plates give body a circular appearance. Mean total lorica length 199.5 μ, lorica width 144.9 μ at widest point. Median dorsal spines (28.5 μ long) recurved ventrally; lateral dorsal spines (19.7 μ) about twice as long as intermediate dorsals (9.6 μ).

Males of *N. michiganensis* have not been observed.

DISCUSSION

Notholca michiganensis, a cold planktonic stenotherm, shows strong morphological resemblance to *N. frigida* of Europe, which is considered as a possible polyploid of *N. squamula* (Ruttner–Kolisko 1974). Total lorica length of *N. michiganensis* (180–220 μ) is considerably smaller than for *N. frigida* (300–400 μ) (ibid.). Variation in body shape of *N. michiganensis* (Fig. 1G–I) may be due partly to the state of contraction of the lorica after fixation with formalin. No wrinkles or indentations on the lorica were ever noted. The ventral and lateral aspects of the lorica of *N. michiganensis* are notably similar to *N. squamula* (Fig. 2). In lakes where *N. squamula* and *N. michiganensis* coexist, individuals of intermediate size were not found (Fig. 3; Table 1). However, I observed a large littoral form of *N. squamula* (lorica length 150–195 μ) which, although somewhat larger, agrees in both shape and habitat with the form found by Carlin (1943) and discussed by Amrén (1964). This form was found in samples at inshore Lake Erie stations near Lorain, Ohio, and in Lake Michigan near Two Rivers, Wisconsin, during spring 1976. The relative lengths of the median, intermediate, and lateral dorsal spines determined for 15 specimens were 19.5, 7.8, and 17.4 μ, respectively. The lorica of this large form (mean length 173 μ, width 133 μ) had wrinkles and indentations which commonly occur on the smaller *N. squamula* form. The lorica of *N. michiganensis* is more constricted immediately below the anterior spines than the large *N. squamula* form, which imparts a more circular appearance to the body (Fig. 1G–H; Fig. 2). In addition, the relative lengths of the anterior spines differ. The general morphology of the lorica places *N. michiganensis* in the *N. squamula* group of closely related species.

Notholca michiganensis occurred at water temperatures between 0.5 and 5.2 C with a peak abundance between 0.5 and 2.5 C. *Notholca squamula* was more eurytopic in its distribution and occurred at temperatures between 0.5 and 17.5 C with a peak abundance between 2.0 and 6.0 C.

Notholca michiganensis has not been observed in the Laurentian Great Lakes but is a potentially valuable indicator of oligotrophy of inland lakes of the region (Gannon and Stemberger 1977). Because the species is apparently restricted to cold oligotrophic waters, it may be found to be a common winter component of the zooplankton in higher latitudes of North America.

Acknowledgments

My sincere thanks to D. Cazden, W. Sharp, and D. Fuller for their assistance in taking measurements, and to J. E. Gannon, who provided essential material and support for completion of the study. Special thanks go to Brit G. Björklund whose valuable suggestions were greatly appreciated. This paper was supported by grants from the Environmental Protection Agency (R-802780 and R-803086) and the National Science Foundation's RANN Program (GI-34898), and represents a contribution from the University of Michigan Biological Station.

AHLSTROM, E. H. 1934. A quantitative study of Rotatoria in Terwilliger's Pond, Put-in-Bay, Ohio. Bull. Ohio Biol. Surv. 61: 1–36.

1936. The deep-water plankton of Lake Michigan, exclusive of the Crustacea. Trans. Am. Microsc. Soc. 55: 286–299.

AMRÉN, H. 1964. Ecological and taxonomical studies on zooplankton from Spitsbergen. Zool. Bidr. Uppsala 36: 209–276.

BJÖRKLUND, B. G. 1972. Taxonomic and ecological studies of species of *Notholca* (Rotatoria) found in sea- and brackish water, with description of a new species. Sarsia 51: 25–66.

CARLIN, B. 1939. Die Planktonrotatorien des Motalaström. Medd. Lunds Univ. Limn. Inst. 5: 1–255.

CHANDLER, D. C. 1940. Limnological studies of western Lake Erie, I. Plankton and certain physical-chemical data of the Bass Islands region, from September, 1938 to November, 1939. Ohio J. Sci. 40: 291–336.

DAVIS, C. C. 1954. A preliminary study of industrial pollution in the Cleveland Harbor area, Ohio, III. The zooplankton, and general ecological considerations of phytoplankton and zooplankton production. Ohio J. Sci. 54: 388–408.

1962. The plankton of the Cleveland Harbor area of Lake Erie in 1956–1957. Ecol. Monogr. 32: 209–247.

FORBES, S. A. 1883. The first food of the common whitefish (*Coregonus clupeaformis* Mitch.). Ill. Nat. Hist. Surv. Bull. 1(6): 87–99.

GANNON, J. E., AND R. S. STEMBERGER. 1977. Rotifer and crustacean zooplankton species composition and community structure as indicators of water quality. *In* Symp. on Plankton and Periphyton as Indicators of Water Quality. Environ. Prot. Agency, Cincinnati, Ohio. (In press)

JENNINGS, H. S. 1896. Report on the Rotatoria — with description of a new species, p. 85–93. *In* H. B. Ward [ed.] A biological examination of Lake Michigan in the Traverse Bay region. Bull. Mich. Fish. Comm. 6: 99 p.

NAUWERCK, A. 1972. Notes on the planktonic rotifers of Lake Ontario. Great Lakes Biolimnol. Lab., Can. Cent. Inland Waters. 37 p. (Mimeo)

RUTTNER-KOLISKO, A. 1974. Plankton rotifers: biology and taxonomy. Binnengewaesser Suppl. 26(1): 146 p.

STEMBERGER, R. S. 1974. Temporal and spatial distributions of planktonic rotifers in Milwaukee Harbor and adjacent Lake Michigan. Proc. Conf. Great Lakes Res. 17: 120–134.

NOTES

Volatile Ketones and Alcohols of Codworms (*Terranova decipiens*) from Axenic Culture and Fresh Fish Muscle

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ACKMAN, R. G. 1976. Volatile ketones and alcohols of codworms (*Terranova decipiens*) from axenic culture and fresh fish muscle. J. Fish. Res. Board Can. 33: 2819–2821.

Simple techniques for the gas–liquid chromatographic analysis of volatile ketones and alcohols from cultures or native codworms, *Terranova decipiens*, or from the axenic culture medium, are described. Three ketones (propanone-2, butanone-2, pentanone-3) and two alcohols (butanol-2, pentanol-3) were observed. The proportion of alcohols to ketones was different in culture medium and in native worms from cod, but the latter were qualitatively and quantitatively similar in fresh cod taken in March and September.

ACKMAN, R. G. 1976. Volatile ketones and alcohols of codworms (*Terranova decipiens*) from axenic culture and fresh fish muscle. J. Fish. Res. Board Can. 33: 2819–2821.

Nous décrivons des techniques simples pour l'étude par chromatographie en phase gazeuse des composés cétone et alcool. Ces techniques sont appliquées à l'analyse soit des vers de la morue, *Terranova decipiens*, cultivés ou sauvages, soit de leur milieu de culture axénique. Trois cétones (propanone-2, butanone-2, pentanone-3) et deux alcools (butanol-2, pentanol-3) furent observés. Le rapport d'alcools à cétones est différent dans le milieu de culture et dans le ver provenant de morues; cependant, ce rapport est qualitativement et quantitativement identique pour des morues fraîchement pêchées en mars et septembre.

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THE chemical identities of certain volatile compounds produced by the codworm *Terranova decipiens* have recently been confirmed as butanol-2, butanone-2, pentanol-3, and pentanone-3 (Ackman and Gjelstad 1975). Comparisons of the relative proportions and actual contents of these chemical materials in cod muscle, and in worms and media from axenic culture (Townsley et al. 1963), were carried out to determine if the culture medium contained an ingredient (not found in cod muscle) responsible for the in vitro production of any of the volatiles. Recent occurrences of humans temporarily acquiring this parasite have been reported (Kates et al. 1973; Chitwood 1970, 1975; Jackson 1975; Myers 1976; Juels et al. 1975). It is not known if the volatile compounds are present in the mammalian (seal) part of the life cycle of the codworm, but since we have observed them in the high temperature range (5–35 C) employed for culture it is possible that this is the case. The possibility of the introduction of stricter regulations on their occurrence in fishery products (Jackson 1975; Cheng 1976) will probably stimulate research on their metabolism in mammals as well as in vitro (Bier 1976). Therefore, I have described some simple techniques that are adequate for the qualitative and quantitative study of the codworm volatiles from culture media or from fish.

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Materials and methods — Gas–liquid chromatography (GLC) was carried out on a metal column 3.7 m (12 ft) 10 ng and 3.2 mm (⅛ inch) outer diameter, packed with Chromosorb, 60–80 mesh, coated with 20% Carbowax 1540. The apparatus was a Wilkins Aerograph fitted with a flame ionization detector (FID). The column temperature was 60 C. The injection port was fitted with a glass liner and held at 80 C. The carrier gas flow of 8 ml/min of helium was achieved with .7 kg/cm^2 (10 psig) pressure, low enough to facilitate headspace (vapor) injection with a Hamilton No. 1002N Teflon-sealed syringe with fixed needle.

Study of the medium containing volatiles from codworm culture was carried out by two techniques. In one, 5 ml medium was gently shaken with 2 ml carbon disulphide (CS_2), in a glass-stoppered centrifuge tube and the bulk of the supernatant aqueous phase was removed, leaving the CS_2 solution. If kept chilled this gave consistent GLC analysis patterns for several weeks. The CS_2 solvent (used as purchased) gave several background peaks due to impurities of which two interfered somewhat with the butanone-2 and pentanone-3 peaks but not with their alcohol analogues. Essentially CS_2 gave no "solvent" peak with the FID detector, although some early baseline perturbation occurred (Douglas and Schaefer 1969; Blades 1970). In the other technique 5 ml medium was poured into a 25-ml Erlenmeyer flask containing 5 g anhydrous sodium sulphate. When the flask was sealed with a rubber serum-vial stopper, headspace samples of 1 or 2 ml then gave clean patterns (Fig. 1), and with strict temperature control several reproducible injections could be obtained in a short period.

A convenient identification technique consisted of injecting 2 ml saturated sodium bisulfite solution into the stoppered flasks, swirling, and allowing 30 min to elapse. The headspace butanol-2 and pentanol-3 were not affected but the peak for butanone-2 was reduced by 90% and that for pentanone-3 by 60%.

It was possible to free codworms of medium by rinsing them with distilled water at room temperature. As an alternative to the more sophisticated and large-scale steam distillation technique used for isolating volatiles cleanly and in quantity (Ackman and Gjelstad 1975), a few worms were ground with distilled water (0.3 ml/worm) in a glass tissue homogenizer, and after light centrifugation 0.005 ml of the clear aqueous phase was injected directly into the GLC unit.

Results and discussion — The headspace blank for axenic medium showed that the ethanol originated in the rubber serum-vial stoppers, presumably from past sterilization steps, and not from the medium. The medium blank showed no volatiles. The propanone-2 (acetone) appeared only in the medium after worm culture, but no propanol-2 was detected. Although different samples of medium gave varying ratios of C_4 to C_5 compounds, always with $C_4 < C_5$, the ratios of butanol-2 to butanone-2, and of pentanol-3 to pentanone-3, were almost always the same, about 1:10. Propanol-2 would thus have been a very minor component if this proportion also held in the C_3 compounds.

The cultured codworms had variable amounts of ketone volatiles, but usually pentanone-3 amounted to between 0.1 and 1 ppm/worm. The medium (examined by sampling the headspace vis à vis headspace over standard aqueous solutions, including Na_2SO_4, of volatiles) had one tenth the concentration of the ketones in worms from that particular medium; it is not known to what degree the rinsing of the worms reduced the level of these volatile and water soluble materials in the worm bodies.

Samples of inshore cod caught near Terrence Bay, N.S., in March and September were brought alive to the laboratory. About 30 min after death they were filleted and codworms and surrounding tissues were examined by the homogenization–direct injection technique. Each worm contained approximately 1 ppm butanone-2, 1.7 ppm pentanone-3, 0.3 ppm butanol-2, and 0.6 ppm pentanol-3. Thus the alcohol to ketone ratio was 1:3 instead of the 1:10 obtained for worms cultured at 35 C. This could result from different nutrients or be a temperature effect. The flesh excised from immediately around the worms contained detectable amounts of the same volatiles in roughly the same proportions, but at a distance greater than 2 cm from a worm no volatiles could be detected by this technique. Apparently the codworm emits these volatiles in the natural state in cod muscle but the rate at which they are moved away by fluid circulation in the living fish is not known. The higher (≫10 ppm) levels found in codworms collected from commercial fish in our recent study (Ackman and Gjelstad 1975) could have been due to the local accumulation of the volatiles of live codworms in cod muscle held several days on ice. Unfortunately the culture experiment was terminated before it could be determined if the accumulation of the volatiles in the medium had an adverse effect on the worms, thus possibly explaining the need for daily changes of medium to maintain activity (Townsley et al. 1963).

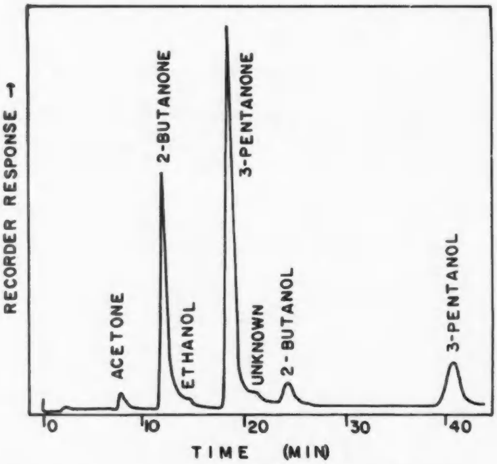

FIG. 1. Headspace sample of volatiles from culture medium analyzed on Carbowax 1540 as described in text. Relative to butanone-2 as 1.00, retention times of other components were as follows: ethanol, 1.24; propanol-2, 1.12; butanol-2, 2.03; pentanol-3, 3.48; propanone-2, 0.65; pentanone-3, 1.56.

The origin and function of these volatile materials is still obscure, but the fundamental biochemical processes of the codworm must be able to utilize materials usually available in animal tissue or broad-based culture media. The amino acids of codworms contain an unusually high proportion of glutamic acid (Freeman et al. 1963). Successive decarboxylations and replacement of the amino group by a keto or hydroxyl group sounds unnecessarily complicated, but could give butanol-2. Steps of these types are not impossible as shown in *Clostridium* biochemistry (Rimerman and Barker 1968) and have been discussed to account for insect alkanones (Blum et al. 1968; Forney and Markovetz 1971).

ACKMAN, R. G., AND R. T. GJELSTAD. 1975. Gas-chromatographic resolution of isomeric pentanols and pentanones in the identification of volatile alcohols and ketones in the codworm *Terranova decipiens* (Krabbe, 1878) Anal. Biochem. 67: 684–687.

BIER, J. W. 1976. Experimental anisakiasis: cultivation and temperature tolerance determinations. J. Milk Food Technol. 39: 132–137.

BLADES, A. T. 1970. The response of carbon disulphide in a flame ionization detector. J. Chromatogr. Sci. 8: 414–415.

BLUM, M. S., F. PADOVANI, AND E. AMANTE. 1968. Alkanones and terpenes in the mandibular glands of *Alta* species (*Hymenoptera, formicidae*). Comp. Biochem. Physiol. 26: 291–299.

CHENG, T. C. 1976. The natural history of anisakiasis in animals. J. Milk Food Technol. 39: 32–46.

CHITWOOD, M. B. 1970. Nematodes of medical significance found in market fish. Am. J. Trop. Med. Hyg. 19: 599–602.

1975. *Phocanema*-type larval nematode coughed up by a boy in California. Am. J. Trop. Med. Hyg. 24: 710–711.

DOUGLAS, D. M., AND B. A. SCHAEFER. 1969. Response of the flame ionization detector to carbon disulphide. J. Chromatogr. Sci. 7: 433–438.

FORNEY, F., AND A. J. MARKOVETZ. 1971. The biology of methyl ketones. J. Lipid Res. 12: 383–395.

FREEMAN, H. C., P. L. HOOGLAND, AND P. H. ODENSE. 1963. The nature of an unusual amino acid-pyramidine complex from the cod parasite, *Porrocaecum decipiens*. J. Fish. Res. Board Can. 20: 1–11.

JACKSON, G. J. 1975. The "new disease" status of human anisakiasis and North American cases: a review. J. Milk Food Technol. 38: 769–773.

JUELS, C. W., W. BUTLER, J. W. BIER, AND G. J. JACKSON. 1975. Temporary human infection with a *Phocanema sp.* larva. Am. J. Trop. Med. Hyg. 24: 942–944.

KATES, S., K. A. WRIGHT, AND R. WRIGHT. 1973. A case of human infection with the cod nematode *Phocanema* sp. Am. J. Trop. Med. Hyg. 22: 606–608.

MYERS, B. J. 1976. The nematodes that cause anisakiasis. J. Milk Food Technol. 38: 774–782.

RIMERMAN, E. A., AND H. A. BARKER. 1968. Formation and identification of 3-keto-5-aminohexanoic acid, a probable intermediate in lysine formation. J. Biol. Chem. 243: 6151–6160.

TOWNSLEY, P. M., H. G. WIGHT, M. A. SCOTT, AND M. L. HUGHES. 1963. The in vitro maturation of the parasitic nematode, *Terranova decipiens*, from cod muscle. J. Fish. Res. Board Can. 20: 743–747.

Incorporation of Fatty Acids into Phosphatidylcholine by Acyl-CoA:1-acyl-*sn*-glycero-3-phosphorylcholine Acyltransferase in Liver of Rainbow trout, *Salmo gairdneri*

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HOLUB, B. J., J. PIEKARSKI, C. Y. CHO, AND S. J. SLINGER. 1976. Incorporation of fatty acids into phosphatidylcholine by acyl-CoA:1-acyl-*sn*-glycero-3-phosphorylcholine acyltransferase in liver of rainbow trout, *Salmo gairdneri*. J. Fish. Res. Board Can. 33: 2821–2826.

The formation of phosphatidylcholine by acylation of 1-acyl-*sn*-glycero-3-phosphorylcholine with fatty acids was demonstrated in microsomal preparations from the livers of rainbow trout (*Salmo gairdneri*). Maximum incorporation of fatty acids into phosphatidylcholine was highly dependent upon the addition of 1-acyl-*sn*-glycero-3-phosphorylcholine to the incubation medium when activated acids in the form of [^{14}C]acyl-CoA esters were provided. The

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acyl-CoA:1-acyl-*sn*-glycero-3-phosphorylcholine acyltransferase showed a selectivity for unsaturated (oleate) over saturated (palmitate) fatty acids when microsomes were prepared from rainbow trout acclimated to 7 or 15 C and assays conducted at both temperatures. The presence of an acyl-CoA hydrolase in the microsomal preparations is also reported. The possible function of the acyltransferase in rearranging the fatty acid composition of cellular phospholipid when fish are exposed to a change in environmental temperature is discussed.

Holub, B. J., J. Piekarski, C. Y. Cho, and S. J. Slinger. 1976. Incorporation of fatty acids into phosphatidylcholine by acyl-CoA:1-acyl-*sn*-glycero-3-phosphorylcholine acyltransferase in liver of rainbow trout, *Salmo gairdneri*. J. Fish. Res. Board Can. 33: 2821–2826.

La formation de phosphatidylcholine par acétylation de la 1-acyl-*sn*-glycéro-3-phosphorylcholine avec acides gras apparaît dans des préparations microsomiques de foies de truite arc-en-ciel (*Salmo gairdneri*). Le maximum d'incorporation d'acides gras dans la phosphatidylcholine dépend fortement de l'addition de 1-acyl-*sn*-glycéro-3-phosphorylcholine au milieu d'incubation quand des acides activés sous forme d'esters de [^{14}C]acyl-CoA sont ajoutés. L'acyl-CoA:1-acyl-*sn*-glycéro-3-phosphorylcholine acyltransférase manifeste une préférence pour les acides gras insaturés (oléates) sur les acides gras saturés (palmitates) quand les microsomes sont préparés à partir de truites arc-en-ciel acclimatés à 7 ou 15 C et dans des essais biologiques effectués aux deux températures. On signale également la présence d'une acyl-CoA hydrolase dans les préparations microsomiques. Nous analysons la fonction possible de l'acyltransférase dans le réarrangement de la composition en acides gras des phospholipides cellulaires quand les poissons sont exposés à un changement de température du milieu.

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It is a well-recognized biological phenomenon that the degree of fatty acid unsaturation of the constituent lipids from fish tissues increases when the environmental temperature is lowered (Roots 1968; Knipprath and Mead 1968; Kemp and Smith 1970). It has been suggested that this biochemical adaptation to temperature reflects a need for such ectothermic organisms to maintain a particular fluidity within cellular membranes (Johnston and Roots 1964). Membrane fluidity is regulated in part by the fatty acid composition of the phospholipids which are structural components of biological membranes (Stoeckenius and Engelman 1969; Reinert and Steim 1970) and control such processes as cellular transport and the activities of membrane-associated enzymes (Hazel and Prosser 1974). Since lecithin or phosphatidylcholine (1,2-diacyl-*sn*-glycero-3-phosphorylcholine) is the most common phospholipid found in fish (Lee and Sinnhuber 1972), we have been interested in elucidating the metabolic pathways responsible for the biosynthesis of this lipid in fish and to assess its role in biochemical adaptation to environmental temperature. We previously documented the presence of CDP-choline:1,2-diacyl-*sn*-glycerol cholinephosphotransferase in liver microsomes of rainbow trout (*Salmo gairdneri*), which provides for the de novo biosynthesis of lecithin, and proposed possible mechanisms by which the CDP-choline pathway may regulate the fatty acid composition of membrane lecithin during temperature adaptation in fish (Holub et al. 1975). Recently, experimental support for this latter concept has been forthcoming in goldfish (*Carassius auratus*) (Leslie and Buckley 1976).

It has also been speculated that the acylation of lysolecithin (1-acyl-*sn*-glycero-3-phosphorylcholine) with long-chain fatty acids may provide for the entry of unsaturated fatty acyl chains into lecithin during cold-temperature acclimation (Anderson 1970; Kemp and Smith 1970), although the existence of this reaction in fish has not been established. The enzymatic hydrolysis of lecithin to lysolecithin has been demonstrated in the microsomal fraction of muscle from rainbow trout (Jonas and Bilinski 1967). Therefore, the present investigations were conducted so as to test for the occurrence of lysolecithin acyltransferase (acyl-CoA:1-acyl-*sn*-glycero-3-phosphorylcholine acyltransferase) in rainbow trout and to examine the activity of this enzymatic reaction in fish acclimated to different temperatures.

Materials and methods — The unlabeled coenzyme A thiolester derivatives (palmitoyl- and oleoyl-CoA) were obtained from the Grand Island Biological Co. [1-^{14}C]plamitoyl-CoA and [1-^{14}C]oleoyl-CoA, having radiochemical purities >99%, were purchased from the New England Nuclear Corp. and diluted with the unlabeled acyl-CoA esters to obtain the desired specific activity (2.6–3.0 × 10^8 counts/min per μmol). The concentrations of adenine and thiol ester in the acyl-CoA preparations used were calculated from the absorbances at 260 and 232 nm, respectively (Seubert 1960). The concentration of the thiol ester was confirmed by measuring the amount of free CoA released when aliquots of these preparations were hydrolyzed with pancreatic lipase (Barber and Lands 1971). 1-acyl-*sn*-glycero-3-phosphorylcholine (1-acyl-GPC) was prepared by hydrolysis of egg lecithin with phospholipase A_2 (Brandt and Lands 1967).

Rainbow trout weighing an average of 10.4 g were used in the experiments. They were maintained at 7 or 15 C and fed a diet similar to diet No. 5 previously described (Cho et al. 1974) except that soybean meal and tallow represented 20 and 6% of the total, respectively. After a period of 4 wk on the diet at the respective temperatures, fish were killed and liver microsomes prepared essentially as described (Fallon and Lamb 1968; Holub et al. 1975). Microsomal protein was measured by the method of Lowry et al. (1951).

Unless stated otherwise in the text, the acyl-CoA:1-acyl-GPC acyltransferase was measured in a medium containing 50 mM Tris-HCl buffer (pH 7.4), 0.17 mM 1-acyl-GPC, 35 μM [1-^{14}C]acyl-CoA, and 200 μg of microsomal protein in a total volume of 0.5 ml. The reaction was initiated by the addition of microsomal protein and quickly terminated by adding 7.5 ml of chloroform–methanol (2:1 vol/vol) after incubating for 2 min at 7 or 15 C. A steady increase in the amount of product formed with time can be maintained for incubation times of only a few minutes in duration. The lipids were immediately extracted (Folch et al. 1957) and subjected to thin-layer chromatography in diethyl ether–formic acid (99:1, vol/vol) followed by chloroform–methanol–acetic acid–water (50:20:5:2, by volume) as described (Wittels 1973) after the addition of unlabeled carrier lipids where required. The radioactivity present in total lipid extracts and in lipid fractions isolated by thin-layer chromatography was measured as outlined (Holub et al. 1975). Suitable aliquots of the lipid extracts in chloroform were evaporated to dryness before addition of the scintillation fluid. Aliquots of the aqueous phase were counted in Aquasol (New England Nuclear Corp.) and gave identical efficiences to those of the lipid extracts. The rate of lecithin synthesis by acyl-CoA:1-acyl-GPC acyltransferase activity was calculated from the specific activity of the acyl-CoA derivatives. Control experiments that involved multiple incubations gave reaction rates ±15% of the mean value. The activity of the microsomal acyl-CoA hydrolase was assessed in a similar fashion by monitoring the appearance of radioactivity in the free fatty acid fractions when radioactive acyl-CoA derivatives were employed as substrates.

Results — Since the radioactive acyl-CoA compounds added to the incubation media are soluble in the aqueous (upper) phase of total lipid extracts, no more than 1.5% of the total radioactivity was found in the chloroform (lipid) phase in control (zero time) incubations and no significant radioactivity above background was detected in the lecithin fraction (Table 1). Approximately 0.6–1.2% of the radioactivity associated with the added [1-^{14}C]palmitoyl-CoA and [1-^{14}C]oleoyl-CoA solutions was recovered as free fatty acid and likely represents a nonenzymatic hydrolysis of these esters to the free acid and free CoA. Incubation of labeled acyl-CoA esters in the presence of the microsomal preparations resulted in a marked increase in the amount of radioactivity associated with the total lipid extracts. The amount of radioactivity incorporated into the lecithin fraction was enhanced by more than fivefold when 1-acyl-GPC was added to the incubation media. The radioactivity appearing in the lecithin fraction in the absence of added 1-acyl-GPC probably represents the presence of endogenous lysolecithins in the microsomal preparations. The occurrence of acyl-CoA hydrolase activity in liver microsomes is supported by the appearance of significant radioactivity in the free fatty acid fraction during the incubations. In contrast to the results on lecithin formation, the amount of radioactivity accumulated in the free fatty acids was not influenced by exogenous 1-acyl-GPC.

The rates of reaction were considerably greater in all cases for oleyl- as compared with palmitoyl-CoA regardless of the acclimation temperature of fish from which the microsomes were derived or the assay temperature (Table 2). For all experimental conditions, the ratio of acyltransferase activities for oleate to palmitate (unsaturated acid to saturated acid) was 1.44 ± 0.07 (mean ± SE). Reaction rates at an assay temperature of 7 C were 37–46% less than those measured at 15 C. Liver microsomes isolated from trout acclimated to 7 C gave acyltransferase rates only slightly lower (2–22%) than those for microsomes derived from fish acclimated to 15 C.

In contrast to the data on acyl-CoA:1-acyl-GPC acyltransferase, the microsomal acyl-CoA hydrolase showed no preference for oleate relative to palmitate (Table 3). For all experiments, palmitoyl-CoA gave reaction rates 13.8 ± 4.6% (mean ± SE) greater than those for oleate. Liver microsomes from fish acclimated to 7 C had hydrolase activities 91–114% those for fish acclimated to 15 C. Measurement of all enzyme activities at an incubation temperature of 7 C gave rates 18–34% lower than those observed at 15 C.

Discussion — Fatty acid analyses and isotopic studies in vivo on fish acclimated to different temperatures have led to speculation that the lysolecithin acyltransferase enzyme(s), if present in fish, might possibly contribute to adjusting the fatty acid composition of cellular lecithin in response to environmental temperature (Anderson 1970; Kemp and Smith 1970). The present inves-

TABLE 1. The acylation of 1-acyl-*sn*-glycero-3-phosphorylcholine with [1-^{14}C]palmitoyl-CoA and [1-^{14}C]oleoyl-CoA in liver microsomes of rainbow trout (*Salmo gairdneri*). FFA = Free Fatty Acids.

Incubation conditions	Counts/min recovered in lipid phase	% distribution of counts/min among lipid fractions				Counts/min in lecithin
		acyl-CoA	lecithin	FFA	other	
Complete system, 15 C[a]	14,847	1.8	49.0	48.4	4.4	7,275
Complete system minus 1-acyl-GPC, 15 C[a]	8,925	2.1	15.9	76.9	5.1	1,419
Complete system, zero time, 15 C[a]	638	11.3	nil	88.7	nil	nil
Complete system 7 C[b]	11,826	1.2	48.4	44.8	5.6	5,724
Complete system minus 1-acyl-GPC, 7 C[b]	7,443	0.8	12.4	75.9	10.9	923
Complete system, zero time, 7 C[b]	795	56.7	nil	41.1	2.2	nil

[a] Incubations were conducted as given in Materials and Methods at 15 C using microsomes from fish acclimated to 15 C. All incubations contained 35 μM [1-^{14}C]palmitoyl-CoA at a specific activity of 2.6 × 10^{6} counts/min per μmol.

[b] Incubations were conducted as given in Materials and Methods at 7 C using microsomes from fish acclimated to 7 C. All incubations contained 35 μM [1-^{14}C]oleoyl-CoA at a specific activity of 3.0 × 10^{6} counts/min per μmol.

TABLE 2. Effect of acclimation and assay temperature on the activity of microsomal acyl-CoA:1-acyl-*sn*-glycero-3-phosphorylcholine acyltransferase using different acyl-CoA derivatives.[a]

Acclimation temperature (C)	Rate of lecithin synthesis[b] (*nmoles/mg protein per min*)			
	15 C[c]		7 C[c]	
	plus palmitoyl-CoA	plus oleoyl-CoA	plus palmitoyl-CoA	plus oleoyl-CoA
15	5.60	8.33	3.27	5.22
7	5.35	7.48	3.20	4.07

[a] Microsomal preparations were isolated after pooling the livers from six fish. Microsomal protein yields were 20.3 and 16.9 mg/g liver for fish maintained at 15 and 7 C, respectively.

[b] The rate of lecithin synthesis by acyltransferase activity is reported as the rate of entry of fatty acid into lecithin in the presence of added 1-acyl-GPC minus that incorporated when it was excluded. Rates of fatty acid entry into lecithin when 1-acyl-GPC was excluded were only 16.1–19.5% of those obtained when it was added.

[c] Assay temperatures.

tigations provide support for the occurrence of this enzymatic reaction in liver microsomes of rainbow trout. The incorporation of radioactive fatty acids into phosphatidylcholine was found to be markedly stimulated by 1-acyl-GPC when ^{14}C-labeled acyl-CoA esters were incubated with microsomal preparations. The reaction mechanism is apparently analogous to that recognized for mammalian systems (Lands 1965):

1-acyl-GPC + acyl-CoA → phosphatidylcholine + CoA

The acyl-CoA:1-acyl-GPC acyltransferase showed a striking preference for unsaturated (oleoyl) relative to saturated (palmitoyl) acyl-CoA esters in agreement with the mammalian enzyme (Hill and Lands 1968). The fatty acid specificity of the fish enzyme as reported herein is consistent with the higher proportion of oleate relative to palmitate at the 2-position of lecithin isolated from fish muscle (Menzel and Olcott 1964). Hepatic microsomes from rainbow trout were also found to have considerable acyl-CoA hydrolase activity as reported for microsomal preparations from rat and pig livers (Jezyk and Hughes 1971). Unlike

Table 3. Effect of acclimation and assay temperature on the activity of microsomal acyl-CoA hydrolase using different acyl-CoA derivatives.[a]

Acclimation temperature (C)	Enzyme activity[b] (*nmoles/mg protein per min*)			
	15 C[c]		7 C[c]	
	plus palmitoyl-CoA	plus oleoyl-CoA	plus palmityl-CoA	plus oleoyl-CoA
15	5.82	5.37	4.80	4.10
7	6.62	5.28	4.38	4.20

[a] Microsomal preparations were identical to those described in footnote to Table 2.

[b] Acyl-CoA hydrolase activity was measured as described in Materials and Methods. Enzyme activity was the same in the presence or absence of added 1-acyl-GPC.

[c] Assay temperatures.

the lysolecithin acyltransferase, the microsomal hydrolase from rainbow trout liver showed a slight preference for the saturated acyl-CoA ester. The reaction rates for acyltransferase activity as reported herein at 15 C are somewhat lower than those found for cholinephosphotransferase in hepatic microsomes of rainbow trout maintained under different experimental conditions (Holub et al. 1975).

The occurrence of acyl-CoA:1-acyl-GPC acyltransferase in rainbow trout warrants consideration of this reaction as an alternative site at which the fatty acid composition of lecithin could be regulated. The degree of unsaturation of the fatty acyl chains found in cellular phospholipid in fish is known to increase with a lowering of environmental temperature (Hazel and Prosser 1974). The possible role of the microsomal cholinephosphotransferase, which provides the CDP-choline pathway for lecithin synthesis, in adjusting the fatty acid composition of cellular phospholipid in fish during temperature acclimation has already been documented (Holub et al. 1975; Leslie and Buckley 1976). The acyltransferase could provide for the enrichment of cellular lecithin in unsaturated fatty acids of low melting points during cold temperature acclimation. Under the present conditions of study, the relative specificity of the acyltransferase towards palmitoyl- or oleoyl-CoA derivatives was not significantly influenced by acclimation or assay temperatures although the oleate ester was the preferred substrate. It remains to be established, however, whether or not the preference of the microsomal acyltransferase for polyunsaturated acyl-CoA esters may be influenced by environmental temperature. Furthermore, the availability of unsaturated fatty acids synthesized by way of desaturation reactions in cold-acclimated fish (Ninno et al. 1974) may control the rate of entry of these acids into membrane phosphatidylcholine by such acylation reactions under physiological conditions. The presence of microsomal phospholipase in muscle from rainbow trout (Jonas and Bilinsky 1967) and the acyl-CoA-:1-acyl-GPC acyltransferase provide the basis for the functioning of a possible deacylation–reacylation cycle in fish tissues. The operation of such a cycle could allow the fatty acid composition of the lecithin molecules derived by the CDP-choline pathway to be rearranged in response to changes in environmental temperature so as to maintain the fluidity of biological membranes at a level necessary for normal cellular functions.

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Anderson, T. R. 1970. Temperature adaptation and the phospholipids of membranes in goldfish (*Carassius auratus*). Comp. Biochem. Physiol. 33: 663–687.

Barber, E. D., and W. E. M. Lands. 1971. Determination of acyl-CoA concentrations using pancreatic lipase. Biochim. Biophys. Acta 251: 361–366.

Brandt, A. E., and W. E. M. Lands. 1967. The effect of acyl-group composition on the rate of acyltransferase-catalyzed synthesis of lecithin. Biochim. Biophys. Acta 144: 605–612.

Cho, C. Y., H. S. Bayley, and S. J. Slinger. 1974. Partial replacement of herring meal with soybean meal and other changes in a diet for rainbow trout (*Salmo gairdneri*). J. Fish. Res. Board Can. 31: 1523–1528.

Fallon, H. J., and R. G. Lamb. 1968. Acylation of *sn*-glycerol-3-phosphate by cell fractions of rat liver. J. Lipid Res. 9: 652–660.

Folch, J., M. Lees, and G. H. Sloane Stanley. 1957. A simple method for the isolation and purification of total lipids from animal tissues. J. Biol. Chem. 226: 496–509.

Hazel, J. R., and C. L. Prosser. 1974. Molecular mechanisms of temperature compensation in poikilotherms. Physiol. Rev. 54: 620–677.

HILL, E. E., AND W. E. M. LANDS. 1968. Incorporation of long-chain and polyunsaturated acids into phosphatidate and phosphatidylcholine. Biochem. Biophys. Acta 152: 645–648.

HOLUB, B. J., K. NILSSON, J. PIEKARSKI, AND S. J. SLINGER. 1975. Biosynthesis of lecithin by the CDP-choline pathway in liver microsomes of rainbow trout, *Salmo gairdneri*. J. Fish. Res. Board Can. 32: 1633–1637.

JEZYK, P. F., AND H. N. HUGHES. 1971. Studies on the hydrolysis and utilization of long chain acyl CoA thioesters by liver microsomes. Lipids 6: 107–112.

JOHNSTON, P. V., AND B. I. ROOTS. 1964. Brain lipid fatty acids and temperature acclimation. Comp. Biochem. Physiol. 11: 303–309.

JONAS, R. E. E., AND E. BILINSKI. 1967. Phospholipase A activity in rainbow trout muscle. J. Fish. Res. Board Can. 24: 2555–2562.

KEMP, P., AND M. W. SMITH. 1970. Effect of temperature acclimatization on the fatty acid composition of goldfish intestinal lipids. Biochem. J. 117: 9–15.

KNIPPRATH, W. G., AND J. F. MEAD. 1968. The effect of environmental temperature on the fatty acid composition and on the *in vivo* incorporation of 1-^{14}C-acetate in goldfish (*Carassius auratus L.*). Lipids 3: 121–128.

LANDS, W. E. M. 1965. Lipid metabolism. Ann. Rev. Biochem. 34: 313–346.

LEE, D. J., AND R. O. SINNHUBER. 1972. Lipid requirements, p. 145–180. *In* J. E. Halver [ed.] Fish nutrition. Academic Press, Inc., New York, N.Y.

LESLIE, J. M., AND J. T. BUCKLEY. 1976. Phospholipid composition of goldfish (*Carassius auratus L.*) liver and brain and temperature-dependence of phosphatidyl choline synthesis. Comp. Biochem. Physiol. 53B: 335–337.

LOWRY, O. H., N. J. ROSEBROUGH, A. L. FARR, AND R. J. RANDALL. 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193: 265–275.

MENZEL, D. B., AND H. S. OLCOTT. 1964. Positional distribution of fatty acids in fish and other animal lecithins. Biochim. Biophys. Acta 84: 133–139.

NINNO, R. E., M. A. P. DE TURRENGO, J. C. CASTUMA, AND R. R. BRENNER. 1974. Specificity of 5- and 6-fatty acid desaturases in rat and fish. Biochim. Biophys. Acta 360: 124–133.

REINERT, J. C., AND J. M. STEIM. 1970. Calorimetric detection of a membrane-lipid phase transition in living cells. Science 168: 1580–1582.

ROOTS, B. I. 1968. Phospholipids of goldfish (*Carassius auratus L.*) brain: the influence of environmental temperature. Comp. Biochem. Physiol. 25: 457–466.

SEUBERT, S. 1960. S-palmityl coenzyme A. Biochem. Prep. 7: 80–83.

STOECKENIUS, W., AND D. M. ENGELMAN. 1969. Current models for structure of biological membranes. J. Cell Biol. 42: 613–646.

WITTELS, B. 1973. Acyl coenzyme A: 1-acylglycerophosphorylglycerol acyltransferase from rat liver. J. Biol. Chem. 248: 2906–2911.

Phosphoglucomutase Polymorphism in the Green Crab, *Carcinus maenas*

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SHERBURNE, S. W. 1976. Phosphoglucomutase polymorphism in the green crab, *Carcinus maenas*. J. Fish. Res. Board Can. 33: 2826–2829.

Starch-gel electrophoresis revealed a polymorphic system for the enzyme phosphoglucomutase (PGM) in muscle extracts of 100 green crabs, *Carcinus maenas*, sampled from Boothbay Harbor, Maine, in 1970 and 1971. This system comprised six phenotypes, including three homozygotes and the corresponding heterozygotes. Each phenotype consisted of two or more isozyme bands. The inheritance of the observed phenotypes can be explained by the existence of three allelic autosomal genes, PGM^a, PGM^b, and PGM^c. The PGM isozymes of the green crab are described with gene frequency data from the population at Boothbay Harbor. These data conform to the Hardy–Weinberg distribution.

SHERBURNE, S. W. 1976. Phosphoglucomutase polymorphism in the green crab, *Carcinus maenas*. J. Fish. Res. Board Can. 33: 2826–2829.

L'électrophorèse de gel d'amidon révèle l'existence d'un système polymorphique pour l'enzyme phosphoglucomutase (PGM) dans des extraits de muscle de 100 crabes enragés, *Carcinus maenas*, recueillis à Boothbay Harbor, Maine, en 1970 et 1971. Ce système comprend six phénotypes, y compris trois homozygotes et les hétérozygotes correspondants. Chaque

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phénotype est constitué de deux bandes d'isozymes ou plus. L'hérédité des phénotypes observés peut s'expliquer par la présence de trois gènes autosomiques allélomorphes, PGM^a, PGM^b et PGM^c. Nous décrivons les isozymes de PGM du crabe enragé avec données sur la fréquence des gènes de la population de Boothbay Harbor. Ces données sont conformes à la distribution de Hardy–Weinberg.

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A number of enzymes were utilized at the Boothbay Harbor laboratory in a search for genetic markers in population studies of marine organisms in the northwest Atlantic. One of these enzymes was phosphoglucomutase (PGM), which interconverts glucose 1-phosphate (G1P) to glucose 6-phosphate (G6P), an essential reaction in carbohydrate metabolism. PGM showed genetic polymorphism in the green crab, *Carcinus maenas*, and provided a genetic marker for studies of this species.

This report describes the PGM isozymes of the green crab obtained by starch-gel electrophoresis and gives gene frequency data from the population at Boothbay Harbor.

Materials and methods — A total of 100 individuals of *C. maenas* were captured in standard green crab traps (Welch 1969) fished from the laboratory dock at Boothbay Harbor. Sixteen green crabs were obtained on May 22, 1970 and the remaining 84 crabs were obtained from November 17 to December 8, 1971.

The crabs ranged in width from 60 to 85 mm and consisted of 15 females and 85 males.

Extracts were prepared by grinding hearts and livers in 2 times their volume of distilled water and skeletal muscle in 4 times its volume. Homogenates were centrifuged at 15,000 *g* for 30 min at 1 C. One drop of supernatant was delivered onto a sample wick before insertion in the starch-gel. Sample wicks consisted of 4 × 6-mm pieces of Beckman No. 319329 filter paper.

Horizontal starch-gel electrophoresis was performed using essentially the same methods as Ridgway et al. (1970, 1971) and Barlow and Ridgway (1971). A stock buffer of pH 8.70 was prepared by dissolving 20 g of tris(hydroxymethyl)aminomethane, 3 g of disodium EDTA (ethylenediamine tetraacetate), and 3.8 g of boric acid in sufficient deionized distilled water to make 1 liter. The electrode buffer consisted of 700 ml of the stock buffer used full strength in each electrode tray. The gel buffer consisted of 90 ml of the stock buffer diluted to a final volume of 300 ml with deionized distilled water for each gel. The gels were made with hydrolyzed potato starch (Electrostarch Co., Madison, Wisconsin) at a concentration of 12.7%.

Electrophoresis was conducted in a refrigerator at 4 C for 30 min with direct current at 7.5 V/cm (165 V); the sample wicks were then removed and electrophoresis continued for 4½ h at 16 V/cm (350 V). After electrophoresis the gel was sliced horizontally with a continuous-band stainless steel razor.

The staining mixture was a modification of that described by Lush (1969) with the addition of more magnesium chloride and a-D-glucose-1, 6-diphosphate.

An agar overlay was used to stain the isozyme bands. Fifty milliliters of 0.2 M Tris-HCl staining buffer (pH 8.0) was divided into two 25-ml flasks. Agar (0.5 g) was added to one flask, heated on a stirring hot plate to 75 C, and then allowed to cool to 55 C. The stain ingredients, consisting of 90 mg glucose-1-phosphate disodium, 4 mg triphosphopyridine nucleotide (TPN) monosodium, 3 mg phenazine methosulfate (PMS), 5 mg nitroblue tetrazolium (crystalline grade III NBT), 300 mg magnesium chloride, 15 IU glucose-6-phosphate-dehydrogenase (G6PD), and approximately 0.5 mg a-D-glucose-1, 6-diphosphate ammonium salt crystalline hydrate, were added to the flask containing 25 ml of the stain buffer, mixed, and then poured into the cooled agar solution. The resultant mixture was poured over a gel slice and allowed to cool and harden. After incubation in the dark at 37 C for 1–1½ h, phenotypes were recorded and the gel was photographed.

Results — The PGM enzyme was more active in extracts of heart and skeletal muscles than in hepatopancreas.

When crude tissue extracts of either green crab heart or skeletal muscles were separated by starch-gel electrophoresis and stained for PGM activity, six distinct phenotypic patterns were observed. Each phenotype consisted of two or more isozyme bands. Allelic variants were named according to the mobility of allozyme bands and designated as *AA*, *AB*, *AC*, *BB*, *BC*, and *CC* (Fig. 1). Allelic variants *AA*, *BB*, and *CC* were considered homozygotes. The homozygous types

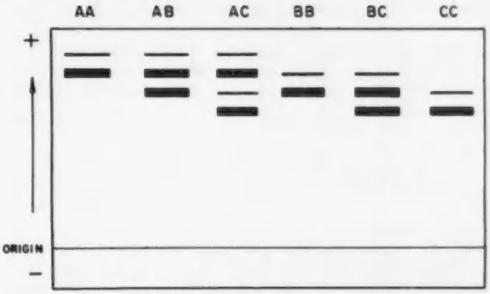

FIG. 1 Starch-gel electrophoretic patterns of the six phosphoglucomutase (PGM) phenotypes observed in the green crab, *Carcinus maenas*.

TABLE 1. Phosphoglucomutase (PGM) phenotype distribution in the green crab, *Carcinus maenas*, from Boothbay Harbor, Maine, assuming Hardy–Weinberg equilibrium.

	Phenotypes							Gene frequencies				
	AA	*AB*	*AC*	*BB*	*BC*	*CC*	Sample size	*PGM*^{a}	*PGM*^{b}	*PGM*^{c}	χ^2	Probability of worse fit (2 *df*)
No. observed	9	17	32	5	19	18	100	0.335	0.230	0.435	0.9767	0.50–0.70
No. expected	11.2	15.4	29.2	5.3	20.0	18.9						

all consisted of two bands; type *AA* had the fastest electrophoretic mobility, type *CC* the slowest mobility, and type *BB* a mobility midway between types *AA* and *CC*. As further evidence that *AA*, *BB*, and *CC* were homozygotes, their respective heterozygous types appeared when extracts of the homozygous types were artificially mixed and subjected to electrophoresis (Fig. 2).

An unidentified, weak-staining brown band was situated approximately 1.5 cm cathodal to each phenotypic pattern. The brown bands contrasted with the dark blue of the phenotypic patterns and were more evident in skeletal muscle extracts than in heart extracts, where the bands were often faint to absent.

An apparent second zone of PGM activity consisting of a single band having essentially the same mobility in each extract was seen in 19 of the 100 samples. This band was approximately 2.5 cm cathodal to the lowest band of the polymorphic system and was the same color as the bands of the polymorphic system.

Statistical analyses showed close agreement of the observed phenotypic frequencies with those expected from the Hardy–Weinberg distribution (Table 1), and contingency tests showed the phenotypes to be independent of sex or carapace width.

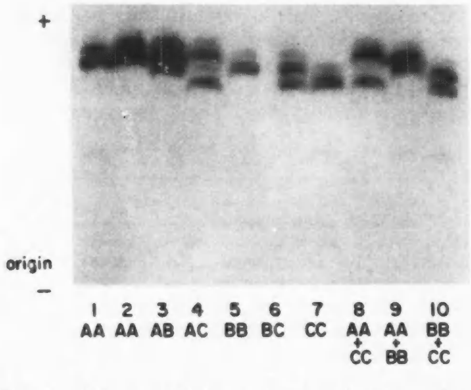

FIG. 2. Green crab, *C. maenas*, phosphoglucomutase (PGM) phenotypic patterns. The six phenotypes observed in this study are included in patterns 1–7. The patterns at positions 8, 9, and 10 are the results of an artificial mixing of the respective homozygote homogenates of positions 2, 5, and 7.

Since heterozygotes were found in both sexes, the *PGM* locus is evidently autosomally inherited. The inheritance of the observed phenotypes can be explained by the existence of three allelic autosomal genes designated as *PGM*^{a}, *PGM*^{b}, and *PGM*^{c}.

Discussion — The sites of activity of PGM in the green crab agreed with the findings of Dawson and Jaeger (1970), who reported that the usual patterns of PGM enzyme levels in the various tissues of animal species they examined was highest in muscle, intermediate in liver, and lowest in blood (with the exception of mackerel blood).

Rees (1963) reported that prior to 1900 the green crab occurred from Cape Cod southward to New Jersey, but had gradually moved northward in response to the trend towards milder winters in the past 50 yr and was now found as far north as Nova Scotia. Welch (1969) reported that since the mid-1950s there has been a great decline in the abundance of green crabs in northeastern Maine while abundance has fluctuated widely in southwestern Maine and Massachusetts. Welch also reported that the general abundance and commercial catch of the soft-shell clam, *Mya arenaria*, a common prey of the green crab, increased markedly during the period of decline of the green crabs. He reported that the declining abundance of green crabs in northern New England was associated with particularly cold winters and a cooling trend in the water.

The green crab, with its marked fluctuations in abundance correlated with changes in ocean climate, seems to be a particularly advantageous animal for studies on possible selective mechanisms. An investigation of the amount of polymorphic variation in the phosphoglucomutase isoenzyme system of green crabs throughout their environmental and geographical limits of distribution may be useful in testing basic hypotheses regarding the mechanisms whereby such polymorphisms are maintained in nature, i.e. selection or random fixation (Lewontin and Hubby 1966; Johnson 1974).

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Barlow, J., and G. J. Ridgway. 1971. Polymorphisms of esterase isozymes in the American lobster (*Homarus americanus*). J. Fish. Res. Board Can. 28: 15–21.
Dawson, D. M., and S. Jaeger. 1970. Heterogeneity of phosphoglucomutase. Biochem. Genet. 4: 1–9.
Johnson, G. B. 1974. Enzyme polymorphism and metabolism. Science 184: 28–37.
Lewontin, R. C., and J. L. Hubby. 1966. A molecular approach to the study of genic heterozygosity in natural populations. II. Amount of variation and degree of heterozygosity in natural populations of *Drosophila pseudoobscura*. Genetics 54: 595–609.
Lush, I. E. 1969. Polymorphism of a phosphoglucomutase isoenzyme in the herring, *Clupea harengus*. Comp. Biochem. Physiol. 30: 391–395.
Rees, G. H. 1963. Edible crabs of the United States. U.S. Fish Wildl. Serv. Fish. Leafl. 550: 10–11.
Ridgway, G. J., R. D. Lewis, and S. W. Sherburne. 1971. Serological and biochemical studies of herring populations in the Gulf of Maine. Special meeting of the biochemical and serological identification of fish stocks, Dublin 1969. Rapp. P. V. Reun. Cons. Int. Explor. Mer 161: 21–25.
Ridgway, G. J., S. W. Sherburne, and R. D. Lewis. 1970. Polymorphism in the esterases of Atlantic herring. *In* Symp. Cytogenet. Fishes. Trans. Am. Fish. Soc. 99: 147–151.
Welch, W. R. 1969. Changes in abundance of the green crab, *Carcinus maenas* (L.) in relation to recent temperature changes. U.S. Fish Wildl. Serv. Fish. Bull. 67: 337–345.

Catastrophe Theory and Fisheries Regulation

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Jones, D. D., and C. J. Walters. 1976. Catastrophe theory and fisheries regulation. J. Fish. Res. Board Can. 33: 2829–2833.

Catastrophe theory is a mathematical approach for analyzing systems that show abrupt changes. This paper attempts to apply the theory to fishery development and collapse, where the key variables are assumed to be stock size, level of economic investment or fleet size, and technological efficiency of the fishery gear. The analysis produces no new predictions about uncontrolled development and its consequences, but it appears to provide a useful way of exploring the implications of regulatory policies involving taxation, development subsidies, and technological improvement.

Jones, D. D., and C. J. Walters. 1976. Catastrophe theory and fisheries regulation. J. Fish. Res. Board Can. 33: 2829–2833.

La théorie de catastrophe est une approche mathématique à l'analyse de systèmes manifestant des changements brusques. Dans cet article, les auteurs essaient d'appliquer cette théorie au développement et à l'affaissement de pêcheries dans lesquelles les variables clés sont considérées comme étant la taille du stock, le niveau d'investissement économique ou taille de la flottille et l'efficacité technologie de l'engin de pêche. L'analyse ne produit pas de nouvelles prédictions quant à un développement non contrôlé et à ses conséquences, mais semble fournir une méthode utile permettant d'explorer les implications de politiques de réglementation touchant la taxation, les subsides au développement et les améliorations technologiques.

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Catastrophe theory was introduced by the French mathematician René Thom (1972), and it is being applied to a startling range of problems in the physical, social, and biological sciences (Zeeman 1976; Clark 1974; Jones 1975). It is a topological approach which can be applied to the analysis of dynamical systems that exhibit large, abrupt changes. A major value is in providing visual models or pictures; dynamical changes are represented as paths or trajectories across graphical surfaces called catastrophe manifolds. In

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three-dimensional cases, as will be analyzed here, points on the graphical surface represent equilibrium values of one ("fast") variable (e.g. stock size) for all possible combinations of the other two ("slow") variables (e.g. fishing effort and technological efficiency). The structure of the surface is inferred from historical observations or examination of the functional relationships that are believed to determine rates of change in the fast variable; folds in the surface represent situations where abrupt change is possible. The theory as applied here should not be viewed as a quantitative tool for making precise predictions; instead it provides a qualitative means for generating "warning signs" concerning large, abrupt changes in system behavior — changes of paramount importance to successful management.

The trend in fisheries research and management today is toward ever greater detail and precision in monitoring and regulation. We would assert that the value of this approach has not been clearly demonstrated; indeed there is a real danger of becoming so buried in detailed, short-term analyses that broad problems and potential dangers are ignored. While they are not a substitute for intensive analyses, approaches like catastrophe theory may provide a way to periodically step back and obtain valuable perspective.

In the following section a simple catastrophe model of fishery development is presented and illustrated with the antarctic whale fishery. The final section uses the model to examine alternative regulatory systems.

Model for fishery development — The first step in catastrophe theory application is to select a small set of variables that best measure the state of the system at any point in time. Initially these variables need not be defined too precisely, nor need they be directly observable or measurable. This latitude is provided by the rules of topology. For a fishing system, the obvious choices are:

1) Stock size, which may be measured in a variety of units and may consist of several geographical substocks or even a mixture of many species.
2) Level of fishing investment as measured by fleet size (the number of fishing boats or harvesting units).
3) Technological and economic efficiency, measured in terms of the catchability coefficient and capacity per boat, and in terms of the lowest stock size for which it would still be economical to continue fishing.

Effort, catch, and catch per effort are deliberately not included as variables, for reasons that will become apparent in the next paragraph.

The next step is to examine components of change in the first variable, stock size, when the other two variables are held fixed. The crudest components of stock change are net production (recruitment plus growth less natural mortality) and catch. Figure 1 shows how we would normally expect these components to be related to stock size for different fixed investment (fleet size) levels and efficiencies. The production component is expected to be a dome-shaped curve, while the catch component will be a saturating curve dependent on economic factors and fishing technology. The low efficiency catch curve has a simple interpretation: for any fixed investment level, there is a stock size P_0 below which it is not economical to fish (effort is zero); above this stock size, catch increases to a limit set by vessel capacities or by catch quotas. The catch at any stock size is higher when fleet size is higher; equilibrium stock P_1 is associated with fleet size I_1, P_2 with I_2, and so forth.

The high efficiency catch curve in Fig. 1 is more interesting. If the investment is low, there is still a single equilibrium stock, P_1. However, when the fleet size is higher (I_2 curve), the system can have three stock equilibria:

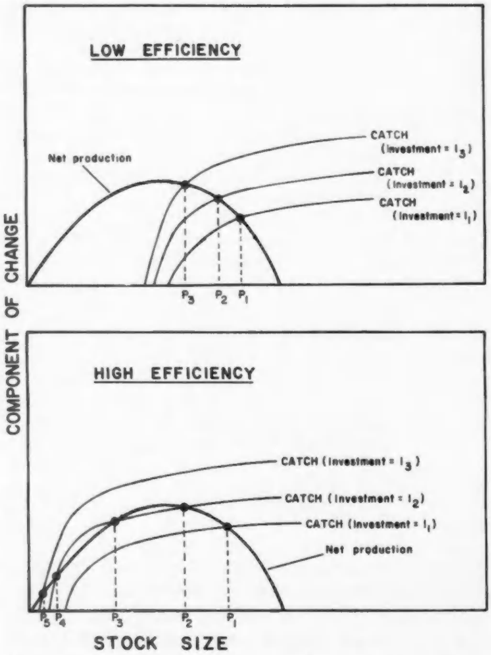

FIG. 1. Aggregate components of stock change; net production and catch for different efficiencies and fleet sizes as measured by investments $I_1 < I_2 < I_3$.

P_2 — Stable in the sense that production will tend to exceed catch for slightly smaller stock size and catch will exceed production for higher stock.

P_3 — Unstable with catches exceeding production when stock drops below P_3, and production exceeding catch above it.

P_4 — Potentially stable as with P_1.

When the fleet size is high (I_3 curve), there is again only one stock equilibrium (P_5) provided that an upper catch limit is not established by quota regulation. Obviously we can envision fish stocks with skewed net production curves such that it would be unlikely for the triple-equilibrium case to exist at all. Also in many fisheries the triple-equilibrium situation may be bypassed entirely or persist for only a short time, if the total catch capacity of the fishing fleet increases rapidly relative to increases in efficiency.

The next step is to rotate the graph axes of Fig. 1 so that equilibrium stock size can be examined as a function of investment and efficiency (Fig. 2). Triple-equilibrium situations then appear as folds in the graph relating equilibrium stock size to investment or fleet size.

The final step is to plot stock equilibria P_1, P_2, etc., for all possible combinations of fleet size and efficiency. These points will form a surface, the catastrophe manifold, as shown in Fig. 3. For low efficiences, the manifold is smooth, indicating a smooth transition in equilibrium stock size as the fleet size is increased. On the other hand, a folded curve appears when the efficiency is high: the three stock equilibria for investment I_2 represent points on the top (P_2), middle (P_3), and bottom (P_4) sheets, respectively. The transition from one to three equilibria (from low to high efficiency) gives rise to the characteristic "cusp catastrophe" predicted by Thom's theory.

We can now begin to explore dynamical changes by tracking stock sizes across the manifold as fleet size and efficiency are allowed to develop over time. It is not necessary to postulate a precise dynamic model for these slow variables, an obvious advantage of this approach. A possible "trajectory" is shown in Fig. 3. Beginning at point "0" (high stock, small fleet, low efficiency), the fishery develops through investment and technological improvement and the stock size decreases. Eventually the fold is reached, and stock size drops catastrophically; in terms of Fig. 1 and 2, this point is reached when the catch curve rises high enough so that the P_2 and P_3 equilibrium points disappear entirely and the only stock equilibrium is at the low level P_5. In any real situation the stock size would not respond so rapidly as to always be on the nearest equilibrium mani-

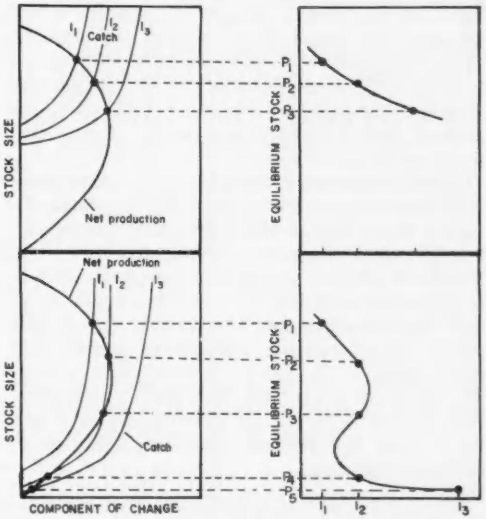

FIG. 2. Changes in equilibrium stock size as a function of investment, as inferred by rotation of the component graphs in Fig. 1.

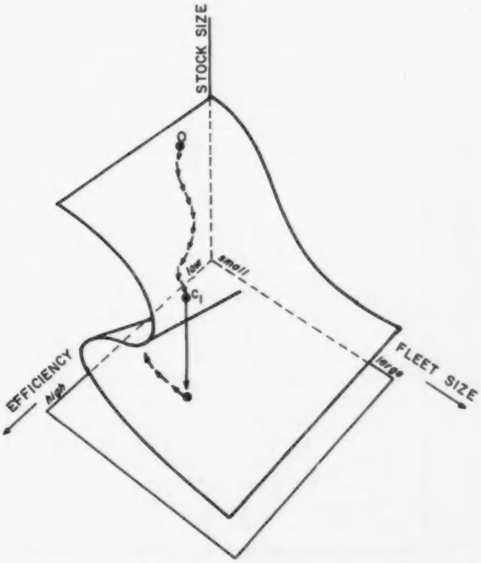

FIG. 3. A catastrophe manifold of equilibrium stock sizes for various combinations of fleet size and efficiency, assuming no fishing regulation. For graphical clarity the fold has been expanded and distorted. Arrows from point 0 show a possible time trajectory. A stock collapse occurs at point C_1.

fold, but the general pattern of response would be preserved unless effort changed very rapidly. After stock size "falls off" the fold, effort drops rapidly in the example trajectory. If the fleet size dropped low enough, the lower fold would be crossed and the stock size would move back towards the upper surface.

Figure 4 shows a projection of the folded manifold region from Fig. 3 onto the fleet size-efficiency plane. The origin of the term "cusp catastrophe" is obvious from the shape of this diagram. The line L_1 represents the fold from Fig. 3 where stock size drops from a high to a low equilibrium; the line L_2 represents the reverse catastrophe where effort decreases enough to permit rapid stock increase. The line L_1 is drawn with a negative slope to reflect the reasonable assumption that smaller fleets are sufficient to trigger a collapse when the efficiency (boat capacity, speed, etc.) is increased.

Application to real fisheries — An interpretation of the antarctic fin whale fishery through catastrophe theory is shown in Fig. 5. The period prior to 1930 was characterized by expanding fishing effort, but relatively low technological efficiency (shore-based processing plants, etc.); there was no clear evidence of catastrophic stock declines until the late 1930s. When the fishery redeveloped after World War II, technological change was rapid, involving factory ships and fleets of highly mobile catcher boats. The catastrophic stock declines during the 1950s and 1960s were characterized, as postulated in Fig. 1, by catches that remained very high until stocks were quite low. In this period the high catches were limited by quotas rather than vessel capacities.

Had effective regulation not been possible, we might now be able to relate the same story for a number of other stocks. Following the transition from bait boats to purse seining, the tropical tuna fisheries have almost certainly moved into a cusp region (as in Fig. 4) where small changes in investment policy or failure to rapidly adjust catch quotas could lead to fishery collapse. The Pacific salmon fisheries for sockeye (*Oncorhynchus nerka*), pink (*O. gorbuscha*), and chum (*O. keta*) are another example, again with the advent of purse seining.

The implications of this line of argument for world fishery development are obvious. If the

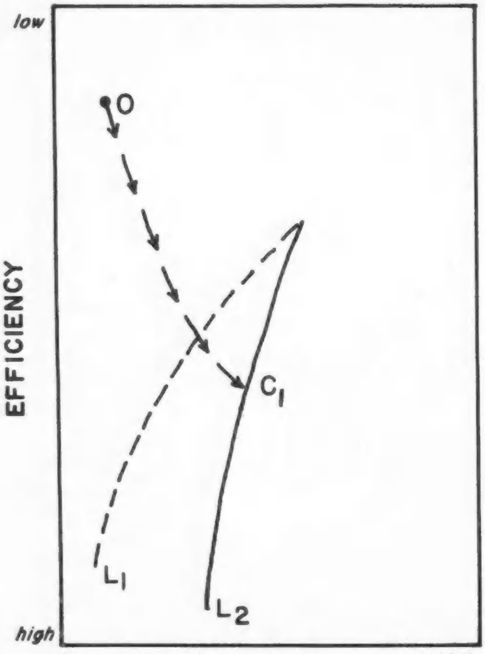

FIG. 4. Projection of the cusp catastrophe manifold from Fig. 3 onto the effort-efficiency plane. Trajectory from Fig. 3 also shown; C_1 represents point when stock collapse occurs.

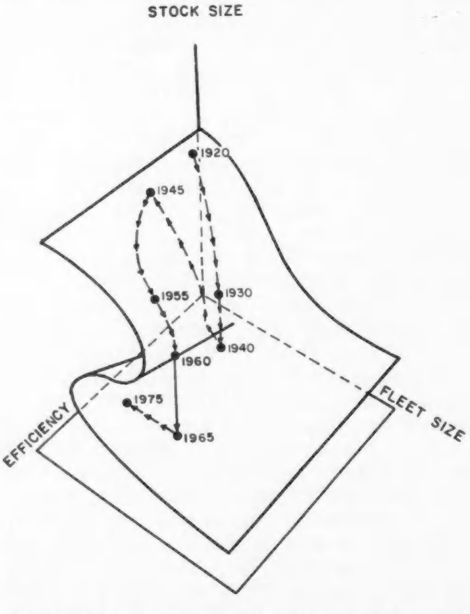

FIG. 5. Changes in antarctic fin and blue whale stocks viewed as a trajectory. The pre-1940 development period moves across the cusp, reflecting a decline just prior to World War II.

catastrophe theory predictions have any basis in reality, we will see increasingly severe problems with fishery regulation as major technological improvements (and government subsidies to lower P_0, Fig. 1) continue. Precise monitoring and control to avoid the folds will become increasingly necessary and difficult, unless we can find other management strategies. The next section addresses this possibility.

Regulatory schemes suggested by the theory — It is necessary to define the kind of policy environment or situation where catastrophe theory might be applied. There is no need for an elaborate theory to deal with cases, like antarctic whaling, where we have already learned the hard way. Rather, we would want to apply the theory in situations where catastrophes have not yet occurred, and where the locations (and even the existence) of folds and cusps are in doubt. For some cases (like salmon and tuna) we may be reasonably sure that the system is now operating within a cusp region, but the distance (across the manifold) to the fold or collapse point will not be known with any precision. What prescriptions can be made in such cases?

Apparently the policy maker may take one of three basic approaches when faced with a system that is believed to contain a cusp catastrophe as shown in Fig. 3–5. Each approach may be accomplished by a variety of tactics, as indicated in the following outline.

Policy 1 — Avoid the cusp region by forcing a reduction in, or maintaining, low fishing efficiency. Tactics:

1) Technological restraints (fishing areas, type of gear, etc.) that reduce the catchability coefficient and thus increase the stock size below which fishing becomes uneconomical.

2) Taxation policies that make it uneconomic to fish when stocks are low; e.g., tax away a fixed percentage of gross earnings.

3) High target escapements that directly drive the catch toward zero even at high stock sizes.

Policy 2 — Broaden the cusp as suggested by Beer and Casti (1975) as so to increase the range of effort levels that can be permitted without dropping off the fold. Tactics:

1) Fix catch quotas further below MSY indicated from production analyses (moves P_2 and P_3 apart in Fig. 1).

2) Stock enhancement (steepens the production curve and moves P_3 to the left in Fig. 1).

3) Fleet size limitations that establish implicit quotas through vessel capacity.

Policy 3 — Narrow the cusp and deliberately operate so as to cross the folds and periodically accept small catastrophes. Tactics:

1) Increase the harvesting capacity per vessel while reducing the number of vessels.

2) Increase the stock size below which fishing is uneconomical or prohibited (see Tactic 1, Policy 1).

The Pacific salmon fisheries have gone through an evolution from policies of Type 1 to the current policies which are of Type 2.

Type 3 policies are essentially an adaptive (Walters and Hilborn 1976) approach to management. The idea is to deliberately manage the system so as to allow minor catastrophic jumps that provide information on the structure of the manifold, without inviting major disasters by trying to maintain perfect stability (Policy 2) or by thwarting technological improvement (Policy 1). Clearly, Type 3 policies offer rich possibilities for future research and management.

The prescriptions outlined above have all been suggested in the fisheries literature, except the Policy 3 approach. Catastrophe theory provides a relatively simple, graphical way to show how they are interrelated; as such it may be a valuable pedagogic tool for analysts who wish to have their ideas heeded by policy makers.

BEER, S., AND J. CASTI. 1975. Investment against disaster in large organizations. IIASA RM-75-16, Laxenburg, Austria. 24 p.

CLARK, C. 1974. Possible effects of schooling on the dynamics of exploited fish populations. J. Cons. Cons. Int. Explor. Mer 36: 7–14.

JONES, D. 1975. The application of catastrophe theory to ecological systems. *In* G. S. Innis [ed.] Simulation in systems ecology. Simulation Counc. Proc. (In press)

THOM, R. 1972. Stabilité structurelle et morphogenese. Benjamin, New York, N.Y. 348 p.

WALTERS, C., AND R. HILBORN. 1976. Adaptive control of fishing systems. J. Fish. Res. Board Can. 33: 145–159.

ZEEMAN, E. C. 1976. Catastrophe theory. Sci. Am. 234: 65–83.

Flatfish Densities Determined with a Diver-Operated Flounder Sampler

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WALTON, J. M., AND N. W. BARTOO. 1976. Flatfish densities determined with a diver-operated flounder sampler. J. Fish. Res. Board Can. 33: 2834–2836.

Densities of four species of flatfish: rock sole (*Lepidopsetta bilineata*), English sole (*Parophrys vetulus*), C-O sole (*Pleuronichthys coenosus*), and the speckled sanddab (*Citharichthys stigmaeus*) were determined by using a flounder sampler designed for use by a scuba diver biologist on sand bottom habitats. Under semicontrolled conditions the overall estimate of flatfish numbers was 91.0 flatfish/1000 m^2 with a 95% confidence interval of ±17.5. Biomass was estimated at approximately 7.4 kg/1000 m^2.

WALTON, J. M., AND N. W. BARTOO. 1976. Flatfish densities determined with a diver-operated flounder sampler. J. Fish. Res. Board Can. 33: 2834–2836.

Nous avons déterminé la densité de quatre espèces de poissons plats : *Lepidopsetta bilineata*, *Parophrys vetulus*, *Pleuronichthys coenosus* et *Citharichthys stigmaeus*, en nous servant d'un échantillonneur de plies conçu pour usage par un biologiste en plongée autonome sur fonds sableux. Dans des conditions semi-contrôlées, l'estimé global du nombre de poissons plats est de 91.0 individus/1000 m^2, avec limites de confiance à 95% de ±17.5. On estime la biomasse à environ 7.4 kg/1000 m^2.

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THE strip census technique for assessment of fish abundance by scuba divers has generally been limited to visual transect methods as utilized by Brock (1954) in Hawaii and Bardach (1959) in Bermuda. This method was necessary because of the difficulties encountered in sampling reef habitats with conventional gear. Sand bottom habitats, however, do not pose these problems and historically have been sampled with otter trawls and beach seines. Adaptation of the visual transect method to the sand bottom habitat can provide quantitative data at relatively low cost and can eliminate some of the bias of conventional methods.

Fager et al. (1966) suggested the use of a fish rake by scuba divers for obtaining information on small-scale distribution patterns and density of small fish and invertebrates. A modification of this concept, called the flounder sampler, has been developed in conjunction with a base line study for an artificial reef project on a nearshore sand flat in central Puget Sound at Edmonds, Washington. The flounder sampler was used to determine density estimates of four species of flounder: rock sole (*Lepidopsetta bilineata*), English sole (*Parophrys vetulus*), C-O sole (*Pleuronichthys coenosus*), and the speckled sanddab (*Citharichthys stigmaeus*). Variability and confidence intervals were calculated from the data. Unidentified flatfish (those ≤10 cm long) were also recorded. Biomass for these species was determined based on length–weight regression equations.

Materials and methods — The flounder sampler (Fig. 1) is a T-shaped device consisting of a wooden handle, 3.2 cm diam and 1 m long, with a perpendicular head made from a 2-m length of 1 cm diam aluminum rod. Aluminum washers, 2.2 cm diam, are threaded on the rod and secured in place with tape every 10 cm along the head. A plastic transect reel is mounted on the handle and holds a braided nylon transect line marked at 10-m intervals. An aluminum stake attached to the end of the line completes the sampler. The diver is also required to carry a slate for recording and a depth gauge for selecting the appropriate depth.

During the experimental sampling dives, the diver proceeded to the maximum depth to be sampled and placed the stake at that depth. The flounder sampler was pushed through the sand at that depth contour, "kicking" out flatfish in a swath 30 × 2 m. The diver recorded the species and estimated lengths by comparing the length of the fish with the 10-cm spacing of washers on the head of the sampler. After a transect was completed, the diver rewound the reel to the stake and made another transect run in the opposite direction. If a current was running, the downcurrent transect was run first to eliminate bias caused by fish moving into the area to feed on organisms dislodged by the diver. This attraction phenomenon was documented by noting fish while rewinding after a transect.

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TABLE 1. Average length, density, and biomass of four species of flatfish as determined by use of the flounder sampler. Overall mean $\bar{x} = 5.46$; variance $\sigma^2 = 6.868$.

Species	Mean no./ transect	Avg length (*mm*)	Density (*no./1000 m^2*)	Biomass (*kg/1000 m^2*)
Lepidopsetta bilineata	2.75	164	45.8	3.51
Parophrys vetula	.42	250	6.9	.99
Pleuronichthys coenosus	.71	250	11.8	2.73
Citharichthys stigmaeus	.21	130	3.5	.10
Unidentified flounder	1.38	53	22.9	.04

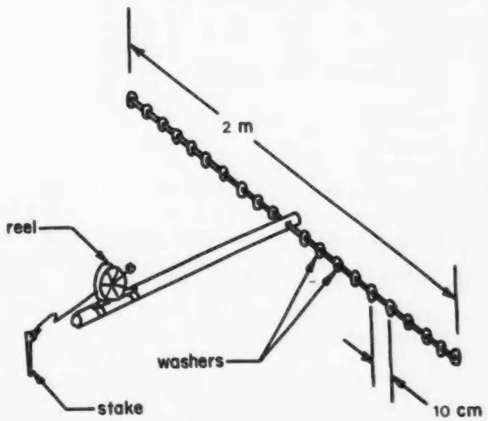

FIG. 1. Flounder sampler for use in determining density estimates of flatfish (detailed description in text).

Three sets of eight transects were completed in August 1975: two sets during a high tide, and one set on a low tide a week later. These tides were selected so that underwater light intensities were approximately equal on each occasion. The first transect set was conducted at a depth of 12.5 m (40 ft) relative to mean lower low water (MLLW), or an actual depth of 15.6 m (50 ft); the second set of 9.4 m (30 ft) depth MLLW, or an actual depth of 12.5 m (40 ft); and the third set was at 12.5 m (40 ft) depth MLLW, on a 0-level tide.

Results — Variances were calculated for each sample set, but no significant differences between sets could be detected ($\alpha = 0.20$) using the *F*-test. To detect differences in the means of the three sets, a square root transformation to normalize the variance was employed. This transformation was selected because of the small numbers in the count data. Analysis of variance again failed to indicate a significant difference ($\alpha = 0.20$) in the means of the three sets. With no probable differences detected, it was assumed that there was no effect due to tide stage or the 3.2-m depth interval at the depths and times tested, and the data could be pooled.

The pooled estimate of the mean numbers of flatfish in a 60-m^2 transect was 5.5 with a 95% confidence interval of 4.4–6.5. In terms of standard density estimates, the number of flatfish per thousand square meters was 91.0 with a 95% confidence interval of 73.5–108.4. Rock sole comprised 50% of the number observed, unidentified flatfish 25%, C-O sole 13%, English sole 7%, and speckled sanddab 4%. By weight, rock sole and C-O sole combined were 85% of the biomass (Table 1).

Fish observed during the rewinding of the transect line were moving into the area just disturbed by the divers. Although these observations suggest that use of a return run along the transect line would not yield accurate density estimates, they do indicate the aggressive behavior of these species. The speckled sanddab and the English sole move in and out quickly, apparently feeding on the organisms kicked into the water column. The rock sole tends to congregate along the transect route and remain in the disturbed area for a longer time.

Discussion — The flounder sampler aids the diver-biologist in making a quantitative assessment of numbers and sizes of bottom fish species present along a measured transect. In this evaluation, every effort was made to reduce the outside sources of variability. All of the transects were made in the same general area by a single biologist on the same type of substrate, within a week's time under similar light intensities. Analysis of transect data between high and low tides and between mean lower low water vs. actual depth did not reveal significant differences in densities at the depths sampled and did not significantly affect the variability. Consequently, the variability of the density estimates using this technique was small, resulting in a narrow confidence interval.

Direct observations of fish behavior and distribution, and more accurate density estimates, are primary advantages of this technique. Unlike otter trawling, an exact distance can be determined and the area to be sampled can be stratified by habitat type. When beach seines are used in deep water, avoidance and escape occur around the edges and over the top, and different habitats are crossed while pulling in the net.

The flounder sampler works best when visibility is at least 2 m. It has been used in 1-m visibility, but it was necessary to employ only one half of the path width. Obviously the estimates of biomass will be affected by the ability of the diver to determine the species and his ability to estimate the fish lengths. Under low visibility conditions, all fish flushed cannot be positively identified, but by noting their presence total densities can still be determined. Recording of size also allows for biomass calculations, although not as accurately as for identified species.

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BARDACH, J. E. 1959. The summer standing crop of fish on a shallow Bermuda reef. Limnol. Oceanogr. 4: 77–85.

BROCK, V. E. 1954. A preliminary report on a method of estimating reef fish populations. J. Wildl. Manage. 18: 297–308.

FAGER, E. W., A. O. FLECHSIG, R. F. FORD, R. I. CLUTTER, AND R. J. GHELARDI. 1966. Equipment for use in ecological studies using Scuba. Limnol. Oceanogr. 11: 503–509.

Trace Metals (As, Cd, Cu, Fe, and Zn) in Arctic cod, *Boreogadus saida*, and Selected Zooplankton from Strathcona Sound, Northern Baffin Island

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BOHN, A., AND R. O. MCELROY. 1976. Trace metals (As, Cd, Cu, Fe, and Zn) in Arctic cod, *Boreogadus saida*, and selected zooplankton from Strathcona Sound, northern Baffin Island. J. Fish. Res. Board Can. 33: 2836–2840.

Arctic cod (*Boreogadus saida*) were captured by bottom trawl at depths of 120–300 m. Zooplankton were obtained by bottom to surface tows from the Arctic cod habitat. Average concentrations of arsenic (As) and zinc (Zn) were higher in Arctic cod fillets than in livers from the same fish; cadmium (Cd) and iron (Fe) concentrations were higher in livers. The difference between copper (Cu) concentrations in muscle and liver was not significant. As and Zn in whole fish, and As in fillets from seven specimens, were positively correlated to body weight. Cu and Fe in whole fish were negatively correlated to body weight, whereas Cd was not related to body weight. Examination of Arctic cod stomach contents indicated that planktonic copepods were important in the diet. Cd levels were 5–8 times higher in the unsorted copepods than in the Arctic cod, whereas As, Cu, Fe, and Zn were higher in the fish than in the copepods.

BOHN, A., AND R. O. MCELROY. 1976. Trace metals (As, Cd, Cu, Fe, and Zn) in Arctic cod, *Boreogadus saida*, and selected zooplankton from Strathcona Sound, northern Baffin Island. J. Fish. Res. Board Can. 33: 2836–2840.

On a capturé des saïdas (*Boreogadus saida*) au chalut de fond à des profondeurs de 120–300 m. On a recueilli du zooplancton dans des traits effectués du fond à la surface dans l'habitat du saïda. Les teneurs moyennes en arsenic (As) et en zinc (Zn) sont plus élevées dans les filets de saïda que dans le foie de mêmes poissons; les teneurs en cadmium (Cd) et en fer (Fe) sont plus élevées dans le foie. La différence de teneur en cuivre (Cu) entre le muscle et le foie n'est pas significative. As et Zn dans les poissons entiers et As dans les filets prélevés sur sept individus montrent une corrélation positive avec le poids corporel. Cu et Fe dans les poissons entiers sont en corrélation inverse du poids corporel, alors que Cd n'a pas de rapport avec le poids corporel.

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L'examen de contenus stomacaux du saïda indique que les copépodes planctoniques sont importants dans le régime. Les niveaux de Cd sont de 5–8 fois plus élevés dans les copépodes non triés que dans le saïda, alors que As, Cu, Fe et Zn sont plus élevés dans les poissons que dans les copépodes.

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IN the development of new mining projects it is important to ensure that waste materials, particularly concentrator tailing and waste rock containing potentially mobile heavy metals, are disposed of in a manner that will lead to minimal environmental contamination. Also, it is now an accepted practice to examine background metal levels in important terrestrial and aquatic organisms in areas where new mining developments will go into production; thus any significant changes in biological metal levels due to mining operations may be detected. This is of great importance in the arctic because a substantial portion of the diet of the indigenous human populations often consists of wildlife which may bioaccumulate noxious materials resulting from any careless or accidental industrial discharge. Unfortunately, the harsh climatic and topographic conditions may severely limit feasible engineering solutions for waste disposal.

Our determination of background metal levels in marine fauna was part of a wider environmental baseline investigation carried out during the summers of 1974 and 1975 on behalf of Nanisivik Mines Ltd., the developers of a zinc–lead deposit in the area. Arctic cod (*Boreogadus saida*), by far the most common teleost species at depths below 100 m, was used to investigate levels of natural metals. Two zooplanktonic components of the Arctic cod diet were also analyzed to compare metal concentrations in the carnivorous Arctic cod with corresponding concentrations one step lower in the food chain. Only metals present at detectable levels in the zinc–lead ore were included in the analyses.

Materials and methods — The study was conducted in Strathcona Sound, a high-arctic fjord extending roughly 50 km east from Admiralty Inlet at approximately 73°11′N, 85°10′W (Fig. 1). The cod were captured by 1½-in. stretch-mesh, semiballoon bottom trawl at depths ranging from 120 to 300 m during two hauls conducted in late August 1975. The trawl was provided with a ½-in. stretch-mesh inner liner in the cod end, to hold small specimens. Plankton samples were collected using a 1 m diam nylon net of 243-μ aperture, equipped with a plastic PVC bucket incorporating a stainless steel mesh screen. The net was lifted vertically to the surface from depths of 5–10 m off the bottom at stations located in the general area of trawling. All samples were hauled on board upwind to the boat and transferred directly into clean plastic containers to avoid the possibility of contamination. The stomach contents of all fish were later removed and examined under the microscope in the field. Sixty of the smallest Arctic cod, all <3.5 cm long, were sorted and grouped to make up 20 samples. Each group had from two to five fish of similar length. Livers from 30 fish, <14.5 cm, were grouped in four samples containing 4, 5, 10, and 11 livers; each group was made up of livers from fish of closely similar lengths. The reason for the grouping procedure was to increase sample amounts to 1 g or more, permitting analysis in the laboratory using standard techniques. Statistically, the respective metal levels in each group were later regarded as representing one single fish whose weight was the mean weight of all fish in the respective group. All fillets, livers, fish carcasses, and whole fish were frozen in individual Whirlpak plastic bags for shipment to the laboratory. Plankton were sorted directly into Whirlpaks for freezing and shipment.

In the laboratory the samples were weighed, dried at 105 C for 48 h, reweighed, and digested until clear in reagent-grade nitric acid. The digests were diluted to 100 ml, or less where only small amounts of sample were available, using triple deionized water. Metal levels, excluding As, were determined by flame atomic absorption using a Perkin–Elmer Model 303 spectrophotometer. Arsenic determinations were based on the technique described by Leblanc and Jackson (1973) with a modification in the initial step of the procedure. Instead of starting with whole tissue, reagent-grade magnesium nitrate, as an ash-aid, was added to aliquot samples of the nitric acid digests, earlier prepared for the absorption

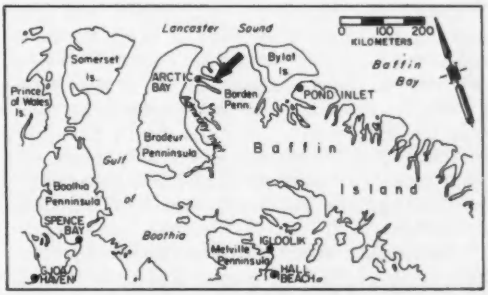

FIG. 1. Study area east of Admiralty Inlet in northern Baffin Island. Arrow indicates proposed area for mining development on the south shore of Strathcona Sound.

TABLE 1. Comparison of metal levels (parts per million dry weight) in fillets and livers from seven individual Arctic cod (*Boreogadus saida*). r = coefficient of correlation to body fresh weight. *Significant at the 99% level.

	Fillets			Livers		
Trace metal	Mean ± SD	Range	r	Mean ± SD	Range	r
As	46±18	26–83	0.903*	4.4±1.1	3.1–6.2	0.583
Cd	<0.5	—	—	0.68±0.25	0.35–0.98	0.559
Cu	4.0±0.90	2.9–5.4	−0.659	3.0±1.0	1.7–4.5	−0.709
Fe	29±8.5	20–41	−0.460	40±7.0	32–53	0.130
Zn	34±4.5	28–39	0.519	19±2.7	15–23	0.647

spectrophotometry. The mixtures were evaporated under an infrared lamp, and dry-ashed as prescribed at 550 C. The residues were dissolved in 6 N reagent-grade HCl and As was then determined as prescribed using the silver diethyldithiocarbamate colorimetric method.

All dissecting and handling of samples was carried out using acid-washed equipment rinsed in triple-deionized water, and rerinsed between use on successive samples.

U.S. Natural Bureau of Standards (NBS) standard reference material 1577 Bovine Liver was analyzed in triplicate for Cd, Cu, Fe, and Zn:

	Metal level (*ppm*)				
Metal	1	2	3	Mean	NBS value
Cd	<1	<1	<1		0.27 ± 0.04
Cu	186	185	186	186	193 ± 10
Fe	250	249	275	256	270 ± 20
Zn	128	126	129	128	130 ± 10

Analytical treatment of the standard reference samples was identical to the treatment of the fish samples, except the bovine liver was freeze-dried as specified in the Certificate of Analysis. Arsenic was not determined; As concentration in bovine liver is only about 0.055 ppm and the value is not certified. However, duplicate As determinations on eight separate fish samples indicated that analytical errors were within ±5%.

Results and discussion — As and Zn were consistently higher in fillets than in livers from the same fish. Cu also averaged higher in fillets, whereas Fe levels averaged higher in the livers (Table 1). For Cu the difference was not significant (matched *t*-test, $t = 0.154$ [6 df], $P > 0.1$) but for Fe the difference was significant at the 95% level ($t = 2.566$ [6 df], $0.05 < P < 0.02$). Cd was higher in the livers than in the fillets of all seven fish.

These results differ from observations by Windom et al. (1973) who found that seven out of eight species of Chondrichthyes had higher Zn and Cu levels in the livers than in the muscle. Taylor and Bright (1973), working with the Nassau grouper, *Epinephelus striatus*, found liver to muscle concentration ratios of about 1600:1 and 47:1 for Cu and Zn, respectively. In the seven Arctic cod from which ratios were available, the ratio for Cu was 0.60:1 and for Zn 0.56:1. Zn was approximately 3 times higher in whole fish than in fillets indicating Zn accumulation elsewhere than in liver or muscle. Arsenic had the lowest ratio observed in the Arctic cod (0.13:1) and appeared to accumulate preferentially in muscle.

Arsenic levels in fillets were positively correlated to body fresh weight, significant at the 99% level, whereas Cu, Fe, and Zn were not (Table 1). Cu in 16 liver samples (Table 2) decreased with increasing body weight. In 84 whole fish, concentrations of As, Cu, Fe, and Zn showed highly significant correlation to body weight: As and Zn increased whereas Cu and Fe decreased with increasing body weight. These correlations were significant at the 99.9% level (Table 2). Thus, studies comparing the natural background metal levels in Arctic cod from Strathcona Sound with corresponding metal levels at a future date, should be designed to allow for evaluation of metal levels in relation to age (weight).

When scatter diagrams were plotted showing metal concentrations in whole fish against body weight, the distributions of As, Cu, Fe, and Zn were nonlinear. Log transformation of body weights offered improved linearity of all four distributions. Figure 2 presents the regression of respective metal concentrations in whole fish on the logarithm of body fresh weight. The log transformations increased the numerical value of each correlation coefficient (shown in the figure), except for Cd, which was not related to body weight. The results agree with Vinogradov's (1953) conclusion that Zn levels in fish tissue increase with age. The results also agree with Taylor and Bright's (1973) observations on Zn in liver and muscle of *E. striatus*, but differ from the observations on arsenic both in *E. striatus* and in other reef-dwelling groupers. As far as we know, As levels in whole fish increasing with increasing body weight have not previously been observed for a teleost species. Cd, Cu, and Fe in Arctic

TABLE 2. Metal levels (parts per million dry weight) in Arctic cod livers and whole Arctic cod collected from Strathcona Sound. *r* = coefficient of correlation to body fresh weight; number of samples in parentheses. *Significant at the 99% level; **significant at the 99.9% level.

Trace metal	Livers			Whole fish		
	Mean ± SD	Range	*r*	Mean ± SD	Range	*r*
As	5.6±1.5 (16)	3.1–8.2	0.392	14±5.4 (84)	6.2–38	0.636**
Cd	0.72±0.37 (15)	0.35–1.6	0.015	0.62±0.22 (47)	0.26–1.5	0.044
Cu	3.7±1.1 (16)	1.7–5.4	−0.645*	4.1±1.1 (84)	2.1–7.7	−0.474**
Fe	39±9.0 (16)	29–58	0.031	125±39 (84)	55–240	−0.373**
Zn	19±4.0 (16)	15–29	−0.037	90±19 (84)	56–142	0.430**

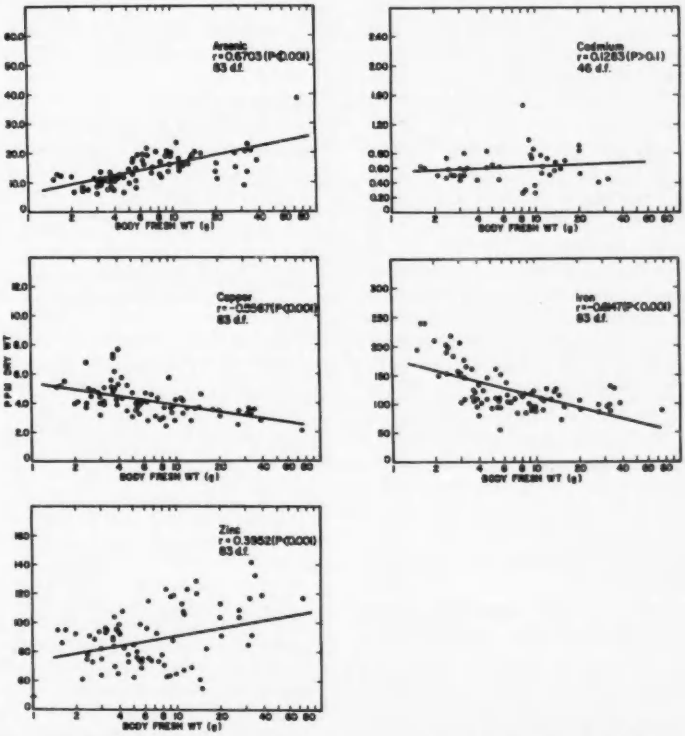

FIG. 2. Regressions of metal levels in whole Arctic cod (*Boreogadus saida*) on body weight; *r* = coefficient of correlation; df = degree of freedom.

cod concur with literature reviewed by Cross et al. (1973), insofar as Cd levels in the Arctic cod remained constant with increasing body weight, whereas Cu and Fe decreased.

Of the 118 Arctic cod stomachs examined, 35 were empty but of 83 with identifiable stomach contents, fish <10 cm long (< about 5 g) contained 65% copepods, whereas fish larger than 10 cm contained only 30% copepods. Pelagic amphipods (family Lysianassidae) were present in smaller quantities (10 and 5%, respectively). Additional stomach contents were chiefly the remains of other small crustaceans, primarily macrurous decapods. Metal levels in plankton from the vicinity of the Arctic cod habitat are presented in Table 3. Arsenic in unsorted copepods measured only about half as high as the average As concentration in whole Arctic cod; Cu, Fe, and Zn also were slightly lower in the copepods. Cd averaged about 5 times higher in

TABLE 3. Metal levels (parts per million dry weight) in zooplankton from the Arctic cod habitat in Strathcona Sound.

	Bottom depth at sampling stn. (*m*)	No. individuals in sample	Trace metal levels				
			As	Cd	Cu	Fe	Zn
Copepods (unsorted)	185	>5000	5.6	5.0	3.7	78	60
" "	295	>5000	7.3	2.8	3.4	75	75
Amphipods (Lysianassidae)	185	10	7.9	7.0	26	87	43
Chaetognaths (*Sagitta* sp.)	185	>200	7.5	1.2	5.6	33	90
" "	295	>200	7.7	1.3	6.3	32	76

copepods than in Arctic cod. Ten planktonic amphipods (family Lysianassidae) captured in the plankton tows were subsequently analyzed for metal levels (Table 3). The amphipods were relatively rich in Cu, but this metal is utilized in their respiratory pigment. Other small pelagic crustaceans were not captured in sufficient quantities to permit metal analyses.

The results on As do not necessarily indicate that this element is biologically magnified across the food chain step leading to Arctic cod from its copepod diet. Assuming that food is the main source of the metal, the results may be due to dietary items, high in As, which at the expense of copepods become increasingly more important in the diet as the fish grow larger. Arsenic levels in four prawns (*Sclerocrangon sharpi*) from the Arctic cod habitat averaged 66.5 ppm (dry weight) and displayed no relation to body weight ($r = 0.0664$) which ranged from 1.6 to 27.9 g (A. Bohn and R. O. McElroy unpublished data). If the similar but smaller, unidentified decapods which were observed with increasing frequency in the stomachs of increasingly larger fish were equally high in As, then the As concentration in whole Arctic cod would approximately equal the average As concentration in the diet. Similarly, the accumulation of As with increasing body weight could reflect an increasing concentration in the diet.

The mean cadmium concentration in copepods from 295 m to surface tow was 2.8 ppm. With an Arctic cod diet containing a minimum of 20% copepods, and assuming that no other Cd was ingested, the minimum average Cd concentration in the diet would be 2.8/5 or 0.6 ppm, corresponding closely to the mean concentration of Cd in whole Arctic cod. Thus Cd in whole Arctic cod did not appear to accumulate above the average Cd concentration in the diet.

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CROSS, F. A., L. H. HARDY, N. Y. JONES, AND R. T. BARBER. 1973. Relation between total body weight and concentrations of manganese, iron, copper, zinc, and mercury in white muscle of bluefish (*Pomatomus saltatrix*) and a bathy-demersal fish *Antimora rostrata*. J. Fish. Res. Board Can. 30: 1287–1291.

LEBLANC, R. J., AND A. L. JACKSON. 1973. Dry ashing technique for the determination of arsenic in marine fish. J. Assoc. Off. Anal. Chem. 56: 383–386.

TAYLOR, D. D., AND T. J. BRIGHT. 1973. The distribution of heavy metals in reef-dwelling groupers in the Gulf of Mexico and Bahama islands. Rep. Dep. Oceanogr., Texas A. and M. Univ., College Station, Tex. 249 p.

VINOGRADOV, A. P. 1953. The elementary chemical composition of marine organisms. Sears Found. Mar. Res., New Haven, Conn. 647 p.

WINDOM, H., R. STICKNEY, R. SMITH, D. WHITE, AND F. TAYLOR. 1973. Arsenic, cadmium, copper, mercury, and zinc in some species of North Atlantic finfish. J. Fish. Res. Board Can. 30: 275–279.

Gonadotropic Hormone Treatment of Rainbow Trout (*Salmo gairdneri*): Plasma Hormone Profile Following a Single Injection[1]

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CRIM, L. W., AND D. M. EVANS. 1976. Gonadotropic hormone treatment of rainbow trout (*Salmo gairdneri*): plasma hormone profile following a single injection. J. Fish. Res. Board Can. 33: 2841–2844.

Immature rainbow trout (*Salmo gairdneri*) were treated with salmon gonadotropic hormone to determine the presence of this hormone in circulation, to describe the plasma profile with time after exogenous hormone treatment, and to determine the difference in plasma gonadotropin levels resulting from treatment with two different hormone doses. The effect of three selected temperatures on the plasma hormone profile was also studied. The plasma concentration of the injected hormone following a low dose (0.02 μg/g) and a high dose (0.2 μg/g) suggested that plasma hormone levels were directly related to the hormone quantity injected. For fish maintained at 6 C, plasma gonadotropin values declined over a period of 7 days reaching low but detectable levels. Lower plasma gonadotropin profiles were observed for fish held at 6 and 15 compared with fish at 2 C ambient temperature. These data suggest the plasma gonadotropin levels and ambient temperature are related in an inverse manner.

CRIM, L. W., AND D. M. EVANS. 1976. Gonadotropic hormone treatment of rainbow trout (*Salmo gairdneri*): plasma hormone profile following a single injection. J. Fish. Res. Board Can. 33: 2841–2844.

Des truites arc-en-ciel (*Salmo gairdneri*) immatures ont été traitées à la gonadotrophine de saumon dans le but de déceler la présence de cette hormone en circulation, de décrire l'évolution dans le temps du profil plasmique après traitement exogène à l'hormone et de déterminer la différence dans les niveaux de gonadotrophine plasmique résultant de l'administration de deux doses différentes d'hormone. On a également étudié l'effet de trois températures sur le profil d'hormone plasmique. La teneur dans le plasma de l'hormone injectée après administration d'une faible dose (0.02 μg/g) et d'une forte dose (0.2 μg/g) donne à croire que les niveaux d'hormone plasmique sont en fonction directe de la quantité d'hormone injectée. Chez des poissons maintenus à 6 C, les valeurs de gonadotrophine plasmique diminuent sur une période de 7 jours pour atteindre des niveaux bas mais décelables. On observe de plus bas profils de gonadotrophine plasmique chez des poissons maintenus à des températures ambiantes de 6 et 15, comparativement à 2 C. Ces données suggèrent que les niveaux de gonadotrophine plasmique sont en relation inverse de la température ambiante.

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TREATMENT of fish with pituitary hormones and in particular gonadotropic hormone continues to be an important method for manipulation of the reproductive cycle. Such procedures ordinarily are used to induce the spawning activities of captive fish. Enhancement of gonadal growth and development is another example where hormone treatment has a practical application, but further study of doses and timing of hormone administration is necessary for this technique to reach its full potential.

Efforts to control the fish reproductive cycle with hormones have suffered from the lack of information regarding hormone uptake and disappearance profiles. Criteria for the success of hormonal control of reproduction have been based upon biological responses of the test animals, e.g. spawning activities. In cases of failure the reasons, such as insufficient dose or frequency of treatment, could only be assumed.

It was decided that the availability of a radioimmunoassay technique suitable for the determination of small amounts (picograms) of gonadotropic hormone provided an opportunity to accomplish the following objectives, using fish with very low levels of gonadotropin in circulation and following intraperitoneal hormone treatment: a) to determine the presence of injected gonadotropin in circulation; b) to estimate the duration of measurable hormone levels present in the blood; and c) to study the influence of selected temperatures on the hormone profile.

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Experimental animals — The fish selected for these studies came from our laboratory stock of rainbow trout (*Salmo gairdneri*) which were maintained in 4–8 C fresh water and fed a daily ration of Ewos no. 5P food pellets. Groups of sexually immature trout 1–2 yr old, average weight 203.2 g (SD = 32.7), were transferred to 227-liter freshwater tanks where the temperature was maintained at the selected level (±1 C) for at least 1 wk in advance of each experimental period. At the beginning of the experiment fish were anesthetized with M.S. 222 (ethyl *m*-aminobenzoate methanesulphonate) (1:5000 dilution), injected with gonadotropic hormone via the intraperitoneal route, identified with a numbered tag inserted alongside the dorsal fin, and returned to the tank for rapid recovery. Food was withheld for the remainder of the experimental period. A total of four 0.5-ml blood samples were drawn from the caudal vasculature at selected times (see below) under M.S. 222 anesthesia using heparinized syringes fitted with 25-gauge needles. Blood samples were placed on crushed ice and the plasma was stored frozen.

Hormone injections — The fish were treated with salmon gonadotropin, SG-100, preparation BCR no. 3, which was purchased from Fisheries and Marine Service, Vancouver Laboratory. The biological potency reported for this material, 0.167 × NIH-LH-S16, was determined by the day old chick testes radiophosphate uptake assay. Dry powdered gonadotropin was freshly weighed and dissolved in Cortlands' Physiological Saline (Wolf 1963) before each experiment. Hormone injections (volume = 0.2 ml/100 g fish wt) were administered at either a low or high dose, 0.02 μg or 0.2 μg/g fish wt, respectively.

Experimental design — Three experiments were conducted at approximately monthly intervals to study plasma gonadotropin profiles in relation to 2, 6, and 15 C ambient temperatures. In each experiment the fish were divided into four subgroups containing 5 (6 C) or 6 (2 and 15 C) individuals as follows: A) control fish receiving no injection and sacrificed on day 0 (day of injection); B) control fish injected with Cortlands' Saline; C) treated fish given the low dose of gonadotropic hormone; and D) treated fish given a high dose of gonadotropic hormone.

Experiments continued for 1 wk following the hormone treatment and blood samples were collected on days 1, 2, 3, and 7. This procedure was changed slightly in the 2 C experiment so that fish were held for 2 wk after hormone injection. The blood sample on day 3 was replaced by a sample collected on day 14.

A fourth and final study was carried out in which six fish per group were treated with either saline or the high gonadotropic hormone dose only. The objective was to study the differences in the plasma gonadotropin profiles for fish held simultaneously at either 2 or 15 C.

Salmon gonadotropin radioimmunoassay (RIA) — The salmon gonadotropin RIA was first reported in 1973 (Crim et al. 1973). Subsequently, minor changes were made in the method to improve its sensitivity and reliability (Crim et al. 1975). Briefly, the double antibody RIA method for salmon gonadotropin depends on competition between radio-labeled gonadotropin (tracer) and "cold" gonadotropin (present in standards or samples) for the limited amount of salmon gonadotropin antibody present. The major advantages of the RIA method are its convenience, sensitivity, and precision.

Results — Plasma gonadotropin in sexually immature rainbow trout is very low. For control fish not injected (Group A) and for fish injected with Cortlands' Saline only (Group B) the plasma gonadotropin values, 0.46 (SD = 0.14) and 0.47 (SD = 0.23) ng/ml, respectively, were not significantly different (Student's *t*-test). These values for gonadotropin were very close to the limit of detection by RIA, 0.30 ng/ml.

Figure 1 presents the plasma gonadotropin profiles for fish treated with hormone and maintained at 2, 6, or 15 C (experiments 1–3). For the selected blood sampling periods, all profiles

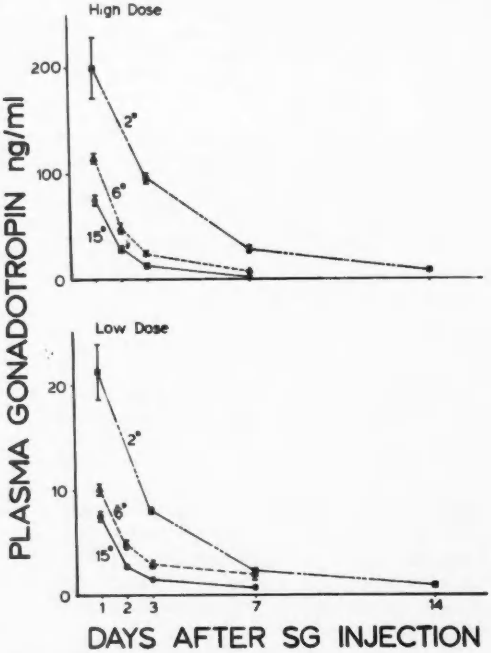

FIG. 1. Plasma gonadotropin profiles for trout maintained at 2, 6, or 15 C and injected with salmon gonadotropin at either a high or low dose level (experiments 1–3). Each point represents mean ± SE from 5 (6 C) or 6 (2 and 15 C) individuals (standard errors not shown are too small to be presented at this scale).

TABLE 1. Plasma gonadotropin ratios[a] for rainbow trout (*Salmo gairdneri*) injected with salmon gonadotropin at two dose levels.

	Blood sample collection					
	Days					
Group	1	2	3	7	14	Avg ratio
2 C	9.4	12.2	—	13.2	10.1	11.2
6 C	11.4	10.6	8.9	4.4	—	8.8
15 C	10.2	11.4	9.5	3.6	—	8.7

[a]Ratio computed by dividing the average plasma gonadotropin value for fish receiving high dose of hormone by the average plasma gonadotropin value for fish receiving low dose of hormone.

showed the highest hormone values 1 day after gonadotropin treatment. These profiles declined thereafter but gonadotropin remained detectable after 7 days. For fish maintained at 2 C, measurable levels of plasma gonadotropin were observed after 14 days.

In each of the three different temperature experiments the fish were treated with either a low or a high dose of salmon gonadotropin. The difference between hormone doses was tenfold and this was reflected in the resulting plasma levels (Table 1). Plasma gonadotropin ratios, computed from the four values within each temperature experiment, were 11.2 (2 C), 8.8 (6 C), and 8.7 (15 C) fold. The overall mean (combined values from all three temperature experiments) value was 9.6 fold.

The plasma gonadotropin profiles were influenced by the ambient temperature of the fish. Figure 1 shows that increasing ambient temperature resulted in lower plasma gonadotropin values for all selected sampling periods over the 7 days following hormone treatment.

A final study (experiment 4) was conducted to investigate the differences in the plasma gonadotropin profiles for fish maintained simultaneously at 2 or 15 C (Fig. 2). Plasma gonadotropin values for fish maintained at 15 C were markedly lower at all blood sampling times selected for study. The average difference in plasma gonadotropin concentration (2 C values divided by 15 C values) for all sampling times was 5.5fold.

Discussion — This report has described the plasma gonadotropin profiles resulting from single intraperitoneal injections with salmon gonadotropic hormone. From these experiments it has been established that, following exogenous hormone treatment, gonadotropin appears in circulation. Furthermore, the profiles indicate that detectable levels of gonadotropin can be found in the blood for as long as 1 wk following the injection. The plasma gonadotropin profile is modified according to the ambient temperature of the fish. With reference to 2 C, higher ambient temperatures are related to lower plasma gonadotropin profiles. This suggests that ambient temperature and plasma hormone profiles are related in an inverse manner.

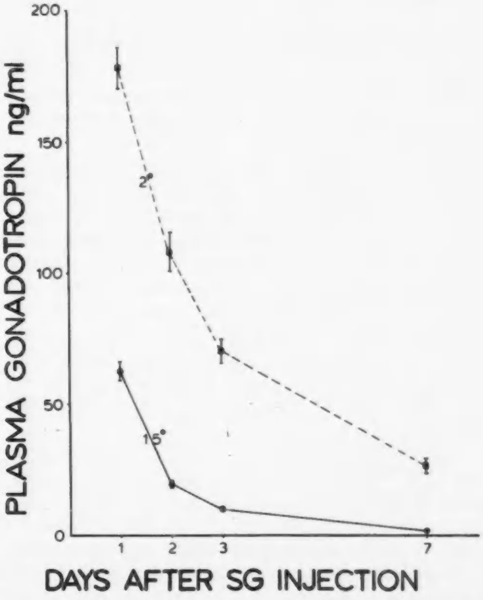

FIG. 2. Plasma gonadotropin profiles for trout maintained at either 2 or 15 C and treated with salmon gonadotropin at the high dose level (experiment 4). Each point represents mean ± SE from six individuals (standard errors not shown are too small to be presented at this scale).

According to the results of this study, treatment of fish with microgram doses of a protein hormone, in this case salmon gonadotropin, develops plasma hormone levels in the nanogram per milliliter range. That this represents physiological levels for fish gonadotropin is suggested by recent studies of Crim et al. (1975) which showed plasma gonadotropin values of a similar magnitude for salmonid fishes at various stages of reproduction. Gonadotropin levels were low during gametogenic phases but marked increases were found for spawning fish. Successful gonadotropin stimulation of the reproduction process of salmonid fish has been previously reported. Acceleration of initial phases of ovarian development in pink salmon was observed after several months of gonadotropin treatment in microgram quantities (Funk et al. 1973). Somewhat higher doses of salmon gonadotropin were required for induction of precocious sexual maturity in male pink salmon (Funk and Donaldson 1972). Single injections of salmon gonadotropin, 10 μg/g, were successful in inducing ovulation of ayu (Ishida et al. 1972).

It is of interest to determine the difference in plasma hormone levels to be expected from varying doses of gonadotropin administered. In this study, two doses of gonadotropin, 10-fold apart, were utilized. The resulting average plasma gonadotropin difference computed from 12 values (3 experimental temperatures and 4 blood sampling times) was 9.6-fold. These observations, although not extensive, suggest that a linear relationship may be expected between the dose of hormone injected and levels developed in the plasma.

Several studies point out the distinct differences in hormone therapy required for gonad growth and development, as opposed to the support necessary for oocyte maturation and ovluation. A single injection of salmon gonadotropin was sufficient to cause ovulation in catfish (Sundararaj and Anand 1972) whereas prolonged hormone treatment (100 μg) of the hypophysectomized catfish was required to promote gonad growth and development. Such differences in hormone therapy were also apparent from the work concerned with hormonal manipulation of the striped mullet reproductive cycle (Shehadeh et al. 1973; Kuo et al, 1974). Yolk accumulation into oocytes in response to gonadotropic hormone is a slow process compared with oocyte maturation and ovulation. We have shown that hormone profiles resulting from a single gonadotropic hormone injection are relatively short-lived and therefore such treatment would seem most appropriate to support maturation and spawning activities. Injection schedules to develop long-term hormone levels require further study. Current work is in progress to investigate the influence of various injection sites and injection vehicles with this objective in mind.

Crim, L. W., R. K. Meyer, and E. M. Donaldson. 1973. Radioimmunoassay estimates of plasma gonadotropin levels in the spawning pink salmon. Gen. Comp. Endocrinol. 21: 69–76.

Crim, L. W., E. G. Watts, and D. M. Evans. 1975. The plasma gonadotropin profile during sexual maturation in a variety of salmonid fishes. Gen. Comp. Endocrinol. 27: 62–70.

Funk, J. D., and E. M. Donaldson. 1972. Induction of presexual maturity in male pink salmon (*Oncorhynchus gorbuscha*). Can. J. Zool. 50: 1413–1420.

Funk, J. D., E. M. Donaldson, and H. M. Dye. 1973. Induction of precocious sexual development in female pink salmon (*Oncorhynchus gorbuscha*). Can. J. Zool. 51: 493–500.

Ishida, R., K. Hirose, and E. M. Donaldson. 1972. Induction of ovulation in Ayu, *Plecoglossus altivelis*, with salmon pituitary gonadotropin. Bull. Jpn. Soc. Sci. Fish. 38: 1007–1012.

Kuo, C., C. E. Nash, and Z. H. Shehadeh. 1974. A procedural guide to induce spawning in grey mullet (*Mugil cephalus* L.). Aquaculture 3: 1–14.

Shehadeh, Z. H., W. D. Madden, and T. P. Dohl. 1973. The effect of exogenous hormone treatment on spermiation and vitellogenesis in the grey mullet, *Mugil cephalus* L. J. Fish. Biol. 5: 479–487.

Sundararaj, B. I., and T. C. Anand. 1972. Effects of piscine and mammalian gonadotropins on gametogenesis in the catfish, *Heteropneustes fossilis* (Black). Gen. Comp. Endocrinol. (Suppl.) 3: 688–702.

Wolf, K. 1963. Physiological salines for freshwater teleosts. Prog. Fish Cult. 15: 135–140.

Effects of Light and Turbidity on the Reactive Distance of Bluegill (*Lepomis macrochirus*)

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VINYARD, G. L., AND W. J. O'BRIEN. 1976. Effects of light and turbidity on the reactive distance of bluegill (*Lepomis macrochirus*). J. Fish. Res. Board Can. 33: 2845–2849.

Changes in the reactive distance of bluegill (*Lepomis macrochirus*) to various sizes of *Daphnia pulex* were measured at light intensities ranging from 0.70 to 215.3 lx (0.065–20.0 ft-c) and at turbidities ranging from 1 to 30 Jackson Turbidity Units (JTU). Both reduced illumination and increased turbidity caused substantial reduction in the reactive distance of bluegill for all prey sizes, and particularly for large prey. This result should be considered in efforts to determine fish feeding rates in lakes, and may be particularly relevant to vertically migrating zooplankton, or those inhabiting more turbid waters.

VINYARD, G. L., AND W. J. O'BRIEN. 1976. Effects of light and turbidity on the reactive distance of bluegill (*Lepomis macrochirus*). J. Fish. Res. Board Can. 33: 2845–2849.

Nous avons mesuré les changements dans la distance à laquelle des crapets arlequins (*Lepomis macrochirus*) réagissent à des *Daphnia pulex* de diverses tailles, à des intensités lumineuses allant de 0.70 à 215.3 lx (0.065–20.0 pi-c) et à des turbidités allant de 1 à 30 unités de turbidité de Jackson JTU. Une intensité lumineuse réduite et une turbidité accrue causent toutes deux une diminution substantielle de la distance de réaction des crapets arlequins quelle que soit la taille des proies, mais particulièrement pour les grosses proies. On devrait tenir compte de ce résultat quand on essaie de déterminer les taux d'alimentation des poissons dans les lacs, et ceci peut être particulièrement pertinent au zooplancton qui se déplace verticalement ou à celui qui habite dans des eaux turbides.

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RECENT theoretical work on the size-selective nature of planktivorous fish predation (Werner and Hall 1974; Confer and Blades 1975; O'Brien et al. 1976) suggests that prey accessibility, combined with preference on the part of the fish for larger prey (Vinyard and O'Brien 1975) are the factors controlling this selectivity. Accessibility, or the opportunity for a predator to pursue prey, is distinguished from preference, defined as the willingness of a predator to pursue a prey, and from escape which involves the capabilities of both predator and prey. The accessibility of plankton prey to visually feeding fish, particularly in the pelagic regions of lakes where no hiding places exist, is a function of the reactive distance (the greatest distance at which a fish can locate the prey) of the fish to particular prey. Thus, zooplankton that are able to reduce their visibility also reduce their accessibility as prey. In natural settings the diel vertical migration of zooplankton (Hutchinson 1967) which places them in the poorly lighted deep areas of lakes during the day may greatly reduce their visibility to planktivorous predators. Yet, until now, measurements of reactive distance and analyses of the feeding of planktivorous fish have been conducted under bright laboratory lights. The present work was undertaken to investigate the effects of decreasing light intensity and increasing turbidity levels on the ability of bluegill (*Lepomis macrochirus*) to locate their zooplankton prey.

Materials and methods — Determination of the reactive distance of bluegill (*L. macrochirus*) to various sizes of *Daphnia pulex* were made in a long plexiglass aquarium (250 × 20 × 20 cm) in a small windowless room. Light was provided by two 40-W fluorescent tubes hung end to end 42 cm above the tank. Light intensity was reduced by wrapping layers of white paper and black cloth around the tubes. Surface light intensities were measured using a Whitney Submersible Photometer LMT-8B and ranged from 215 to 0.70 lx (20.0–0.065 ft-c). A light intensity of 75,000–95,000 lx is usual at the surface of a clear lake during the day; light intensities approaching 0.48 lx would be found on clear moonless nights.

Turbidities ranging from 1 to 30 JTU were created by mixing various amounts of clay sediments obtained from a local turbid pond into the water of the aquarium. The turbidity was measured using a Hach Turbidity Meter 1860A. At turbidity levels higher than 20 JTU some settling and consequent

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reduction of turbidity during experiments was noted; however, variation was never greater than ± 2 JTU. A turbidity level of 20 JTU is fairly muddy water but often found in estuaries and not uncommon in midwestern lakes.

Fish were acclimated to the various light intensities for at least 24 h prior to experimental trials, and to the different turbidities for several hours in accordance with the observation of Protasov (1970) that dark adaptation of the retina of two marine fish was complete in 30 min, but that complete adjustment of the entire receptor apparatus took several hours. The feeding aquarium was maintained near 24 C. Fish were starved for at least 24 h prior to an experiment, and in preliminary experiments we determined that starved bluegill could eat 25 or more large *D. magna* without changing their reactive distance. The fish averaged 65 mm in length.

Reactive distances were obtained by observing the distance at which a fish first began pursuit of a prey measured against two centimeter scales placed along the bottom of the aquarium. Pursuit is easily distinguishable from the more random and slower search activity of the fish; initiation of pursuit is accompanied by direct orientation of the long axis of the fish toward the prey followed by rapid, uninterrupted swimming directly to the prey.

Experiments were begun by separating different-sized *D. pulex* to the nearest 0.25-mm group. Single prey of various sizes were introduced into the experimental aquarium by placing the prey in an opaque tube located immediately above the water and squeezing a pneumatic bulb attached to the tube. Introduction of the prey was generally done while the fish was faced in the opposite direction, and prey were always introduced at a distance greater than the fish's reactive distance for that prey as determined by preliminary observations. Prey were introduced into the central area of the aquarium more than one reactive distance from the end of the tank. This provided the prey with an appropriate background luminance, and in conjunction with turbidity measurements overcomes most of Whitney's (1969) objection to the measurement of illuminance.

Results — Variation in both light intensity and turbidity affected the reactive distance of bluegill to *D. pulex* prey. At light intensities of 10 lx or greater, reactive distance is maximal and increases with increasing prey size (Fig. 1). This relationship is almost identical to that found by Werner and Hall (1974) for *D. magna* and by Confer and Blades (1975) using *D. pulex* as prey. At light intensities below 10 lx the reactive distance for a given size of prey decreases. A linear relationship between prey size and reactive distance at a given light intensity is maintained at all light levels, but at lower light intensities the slope of the regression line is decreased. At the lowest light intensity measured, 0.70 lx, the slope of the regression line is only slightly greater than zero and the reactive distance for all sizes of prey is about 3.5 cm.

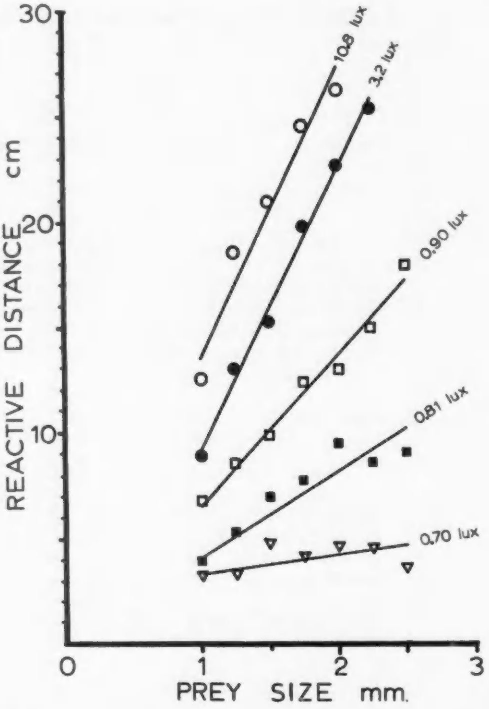

FIG. 1. Relationship between reactive distance and prey size for different illuminances at low turbidity, 1 JTU.

At constant light intensity the reactive distance is greatest at low turbidity levels and diminishes to very low values as the turbidity approaches 30 JTU (Fig. 2). There is again a linear relationship between reactive distance and prey size at different turbidity levels and a decreasing slope with increasing levels of turbidity. At all light intensities at the highest turbidity levels tested, 30 JTU, the slope of the lines is only slightly greater than zero, averaging 1.4, and the reactive distance for all sizes of prey is about 3–4 cm.

Changing light and turbidity levels had much greater impact on the reactive distance to large prey than to small prey (Fig. 3 and 4). These figures also show the threshold nature of the effect of changing light intensities. Above 10.76 lx (1 ft-c) there is little change in reactive distance with increasing light, but below this level there is a rapid decrease in reactive distance.

Multiple regression techniques proved cumbersome in analyzing these data; thus an alternative approach to predicting reactive distance given the light intensity and turbidity level was devised. The technique is based on the observation that

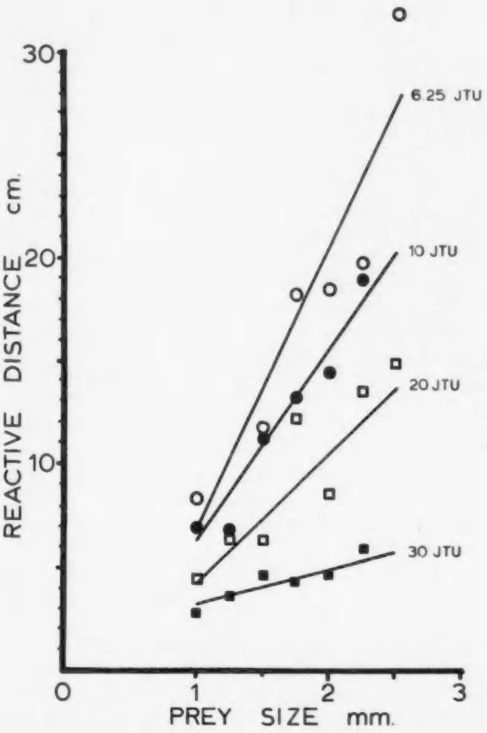

FIG. 2. Relationship between reactive distance and prey size for different turbidities at a constant illuminance, 34.9 lx (3.24 ft-c).

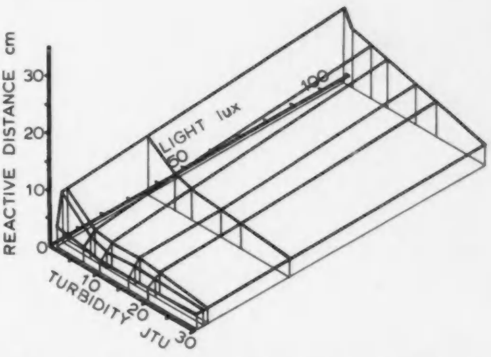

FIG. 3. Three-dimensional plot of bluegill (*Lepomis macrochirus*) reactive distance vs. illuminance and turbidity. These data generated using 1-mm *Daphnia pulex* prey.

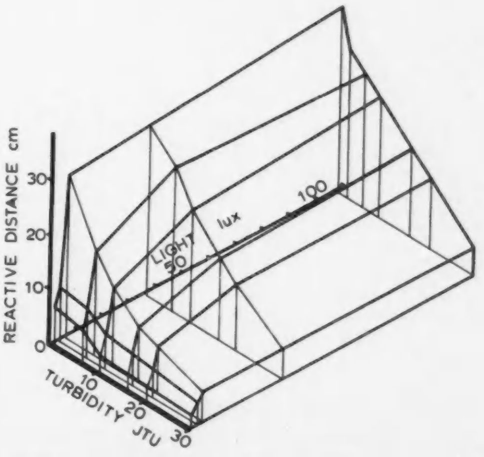

FIG. 4. Three-dimensional plot of bluegill reactive distance vs. illuminance and turbidity. These data generated using 2.5-mm *Daphnia pulex* prey.

the maximum reactive distances under conditions of low turbidity are determined solely by the light intensity and size of the prey, but as turbidity increases this maximum reactive distance is reduced proportionally to a minimum value at turbidities of 30 JTU. Thus the predictive algorithm utilizes the light intensity alone to establish the maximum reactive distance line:

$$\text{Slope} = 5.89 + (-0.29 \times \text{light}) + (19.2 \times \log_{10} \text{light}) \tag{1}$$

where light is the illuminance in lux ($r = .98$).

The predictive equation for the slope of the line describing the relationship between reactive distance and prey size is generated by forcing all the family of straight lines given on Fig. 1 and other data to pass through the origin. Thus changing light intensity alters only the slope and not the intercept of the lines. Turbidity is incorporated by reducing the slope of the maximum reactive distance line by an amount proportional to the turbidity. If the turbidity is 10 JTU the slope of the line is one third the difference between the slope predicted by the light intensity alone and the minimum slope measured at 30 JTU. At a turbidity of 20 JTU the slope would be reduced by two thirds and so on.

This yields an equation that incorporates the effects of both light and turbidity on the reactive distance of sunfish to various prey sizes:

$$\text{RD} = \text{PS} \times \{<[\text{Slope} - \text{MS}] \times [1.0 - (\text{Turbidity}/30.0)]> + \text{MS}\} \tag{2}$$

where RD = reactive distance (centimeters); PS = prey size (millimeters); Slope = slope obtained from equation (1); Turbidity = turbidity of the water in JTU between 0 and 30 JTU; and MS = 1.4, the average slope observed at turbidities of 30 JTU.

This predictive equation is valid between 10 and 0.70 lx and between 1 and 30 JTU, conditions that define the outside maximum and minimum reactive distances observed. Since reactive distance is constant at light intensities equal to or greater than 10 lx and reaches a minimum at light intensities equal to or less than 0.70 lx or turbidities greater than 30 JTU, the values obtained from the equation at these limits are appropriate for use outside the range of the equation.

Discussion — Both increased turbidity and reduced light intensity substantially diminish the reactive distance of bluegill for all prey sizes tested. At high turbidities and/or low light intensities the reactive distance becomes nearly independent of prey size; under such conditions we suspect some other mechanism such as lateral line sensing may become important in detection of nearby zooplankton prey.

The magnitude of the change in reactive distance under different conditions of light and turbidity makes it imperative that these factors be considered when estimating fish feeding rates in a pond or lake. Reduction of reactive distance greatly limits the volume a fish can search in a given time. For example, a 50% reduction in reactive distance reduces the actual volume searched by a factor of 4 if the fish is considered to be searching a cylinder (Confer and Blades 1975), or by a factor of 8 if searching a hemisphere (O'Brien et al. 1976), or a sphere (Werner and Hall 1974).

The relationship between reactive distance and prey escape has not previously been appreciated. A prey that moves out of an attacking predator's reactive distance has in fact escaped, and the predator must begin to search again. Therefore, when the reactive distance is small, as under conditions of low light or high turbidity, the chance of escape, especially for rapid swimming zooplankters such as copepods, is much greater. By contrast, at the high light–low turbidity conditions used in most experimental studies, prey must travel so far to get outside the reactive distance of the fish that escape is almost impossible (Confer and Blades 1975).

At light intensities below 10 lx there was a decline in reactive distance. Wolf and Zerrahan-Wolf (1936) reported that sunfish shift from light to dark adaptation in intensities between 0.097 and 0.010 lx which suggests that the observed decline in reactive distances may not be due to this mechanism. Other reported responses by fish to low light intensities include the observations that the feeding of herring larvae is much reduced at light intensities of 0.97 lx and stops completely at 0.097 lx (Blaxter 1966), and that the feeding rate of jack mackerel drops markedly between approximately 10^{-4} and 10^{-5} mL (Hunter 1968).

Effects of turbidity on the trophic ecology of fish have not received much attention. The activity levels and social behavior of green sunfish (*L. cyanellus*) were reduced and altered in moderately turbid water (14–16 JTU; Hemistra et al. 1969), and total production of bluegill and largemouth bass dropped sixfold in turbid Oklahoma ponds compared to clear ones (Buck 1956). Munz (1958) reported that marine fish living in turbid coastal waters have different absorption maxima in their retinal pigments than do those inhabiting clearer waters.

Examination of Fig. 3 and 4 shows that the relative change in reactive distance with altered light and turbidity levels is much greater for large prey than small prey. If some zooplankton species use the darker, lower portions of lakes as refuges to diminish predation during the day, then the larger species and individuals would be expected deeper in the lake. This thesis corresponds with what is known of zooplankton vertical migration (Hutchinson 1967).

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BLAXTER, J. H. S. 1966. The effect of light intensity on the feeding ecology of herring, p. 393–409. *In* R. Bainbridge, G. C. Evans, and O. Rackham [ed.] Light as an Ecological Factor. Symp. Br. Ecol. Soc., Cambridge. Blackwell Scientific Publications, Oxford.

BUCK, D. H. 1956. Effects of turbidity on fish and fishing. Okla. Fish. Res. Lab. Rep. 56: 62 p.

CONFER, J. L., AND P. I. BLADES. 1975. Omnivorous zooplankton and planktivorous fish. Limnol. Oceanogr. 20: 571–579.

HEMISTRA, N. W., K. D. DAMKOT, AND N. G. BENSON. 1969. Some effects of silt and turbidity on behavior of juvenile largemouth bass and green sunfish. U.S. Dep. Inter., Bur. Sports Fish Wildl. Tech. Pap. 20: 9 p.

HUNTER, J. R. 1968. Effects of light on schooling and feeding of jack mackerel, *Trachurus symmetricus*. J. Fish. Res. Board Can. 25: 393–407.

HUTCHINSON, G. E. 1967. A treatise on limnology. Vol. II. John Wiley and Sons, Inc., New York, N.Y. 115 p.

MUNZ, F. W. 1958. The photosensitive retinal pigments of fishes from relatively turbid coastal waters. J. Gen. Physiol. 42: 455–459.

O'BRIEN, W. J., N. A. SLADE, AND G. L. VINYARD. 1976. Apparent size as the determinant of prey selection by bluegill sunfish (*Lepomis macrochirus*). Ecology (In press)

PROTASOV, V. R. 1970. Vision and near orientation of fish. Acad. Sci. USSR. (Transl. from Russian by the Israel Program for Sci. Transl., Jerusalem, No. 1PST5738)

VINYARD, G. L., AND W. J. O'BRIEN. 1975. Dorsal light response as an index of prey preference in bluegill (*Lepomis macrochirus*). J. Fish. Res. Board Can. 32: 1860–1863.

WERNER, E. E., AND D. J. HALL. 1974. Optimal foraging and the size selection of prey by bluegill sunfish (*Lepomis macrochirus*). Ecology 55: 1042–1052.

WHITNEY, R. R. 1969. Schooling of fishes relative to available light. Trans. Am. Fish. Soc. 98: 497–504.

WOLF, E., AND E. ZERRAHN-WOLF. 1936. Threshold intensity of illumination and flicker frequency for the eye of the sunfish. J. Gen. Physiol. 19: 495–502.

Relationships of Epibiotic Fouling and Mortalities of Eggs of the Dungeness Crab (*Cancer magister*)

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FISHER, W. S. 1976. Relationships of epibiotic fouling and mortalities of eggs of the Dungeness crab (*Cancer magister*). J. Fish. Res. Board Can. 33: 2849–2853.

This study describes the close association of epibiotic microbial fouling on the eggs of *Cancer magister* and egg mortalities. Laboratory experiments showed that nutrient enriched sea water increased the number of measurable filaments on the egg surface and increased the number of egg mortalities. Chemotherapeutic treatment of the sea water decreased the number of filaments and mortalities. The use of antibiotics decreased the number of mortalities while the number of filaments steadily increased, suggesting that antibiotic-sensitive, nonfilamentous forms may be substantially responsible for mortalities caused by microbial fouling. Darkness and ultraviolet irradiation of the sea water had no significant effects on filaments or egg mortalities. Both filamentous fouling and egg mortalities described exponential declines with increased depths into the egg masses.

FISHER, W. S. 1976. Relationships of epibiotic fouling and mortalities of eggs of the Dungeness crab (*Cancer magister*). J. Fish. Res. Board Can. 33: 2849–2853.

L'auteur décrit dans cet article la relation étroite entre les salissures microbiennes épibiotiques sur les œufs de *Cancer magister* et la mortalité des œufs. Des expériences de laboratoire démontrent que l'eau de mer enrichie de substances nutritives cause une augmentation du nombre de filaments mesurables à la surface de l'œuf et une augmentation de mortalité des œufs. Le traitement chimiothérapeutique de l'eau de mer entraîne une diminution du nombre de filaments et de mortalités. L'emploi d'antibiotiques réduit les mortalités, mais cause une augmentation uniforme du nombre de filaments, ce qui donne à croire que des formes non filamenteuses, sensibles aux antibiotiques, peuvent être en grande partie responsables des mortalités résultant de salissures microbiennes. L'obscurité et l'irradiation aux rayons ultraviolets n'ont pas d'effets significatifs sur les filaments ni sur la mortalité des œufs. Les salissures filamenteuses et la mortalité des œufs accusent toutes deux une diminution exponentielle avec augmentation de la profondeur dans les masses d'œufs.

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THE Dungeness crab (*Cancer magister*) fishery has consistently fluctuated in northern California and Oregon but has declined and collapsed in central California since 1960 (Orcutt et al. 1975). A recent study (Fisher and Wickham 1976) showed a greater number of egg mortalities near the mouth of San Francisco Bay than along the rest of the northern California shoreline. Epibiotic fouling by filamentous microorganisms was also found to be increased in the San Francisco region. The study suggested that the egg mortalities could be caused by the epibiotic microorganisms through the restriction of gaseous exchange across the outer egg membrane or through the utilization of oxygen necessary for the developing embryo. It was also suggested that increased nutrient levels in the sea water would produce increased microbial fouling, thereby causing higher mortalities in the crab egg masses.

The following study provides information re-

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garding the effects of various seawater treatments on the number of epibiotic filaments and the number of mortalities in egg masses of the Dungeness crab. The enumeration of filamentous fouling is intended only as an index of overall microbial fouling. Filamentous fouling on egg membranes is normally accompanied by populations of nonfilamentous fouling (unpublished data; Nilson et al. 1975); however, techniques for quantifying these populations of nonfilamentous microbes on egg surfaces have not been determined. The various seawater treatments used in this study were: ultraviolet irradiation to reduce the number of influent microorganisms; nutrient enrichment to enhance microbial growth; antibiotics to retard strictly bacterial growth; darkness to retard the growth of photosynthetic microorganisms; and broad-spectrum chemotherapy to restrict the growth of all these forms.

Materials and methods — The 56 unberried female Dungeness crabs (ranging from 14.8 to 16.2 cm carapace width) used in this study were obtained by the crew of the *N.B. Scofield*, a California Department of Fish and Game research vessel, on September 12, 1975, near the mouth of the Russian River, Sonoma County, California. The crabs were taken to the Bodega Marine Laboratory and held communally in flow-through ambient sea water for 1 wk and fed frozen squid and shrimp 3 times. They were then transferred to individual holding compartments (perforated PVC) in seven 165-liter seawater tanks and were not fed for the remainder of the study. Each tank held 6–10 animals and each compartment was approximately 25 × 25 cm with a 25-cm water depth. Flow rates in each tank were adjusted to 2 liters/min and the tanks were siphoned 3 times/wk to remove accumulated debris.

Egg extrusion in the tanks occurred from September 22 to October 18 with 60% of the extrusions occurring between September 27 and October 7. Although 5–10 cm sand substrate is required for good cementing of the eggs to the abdomen of the female (unpublished data), no substrate was placed in the tanks due to the need for water quality control. As a result, only ⅓–½ of the extruded eggs successfully cemented to the pleopodal setae.

Each of the seven tanks of animals was treated as a separate group according to the following scheme:

Group	Sea water	Treatment
1	Raw	None
2	Filtered, UV, sterilized	None
3	" " "	Nutrient enriched
4	" " " " " "	Malachite green- treated, light
5	" " " " " "	Malachite green- treated, dark
6	" " "	Dark
7	" " "	Antibiotics treated

Raw sea water was passed through 20-mesh silica filtration to remove large debris. Pretreated sea water was additionally passed through successive resin cartridge filters (50-, 25-, and 5-μ mesh) and through an ultraviolet irradiation unit (2–40-W UV lamps in quartz housing).

The sea water for Group 3 was continually treated with dissolved sodium nitrate ($NaNO_3$) and sodium phosphate ($NaH_2PO_4 \cdot H_2O$) with the intent of maintaining these levels at 10 times the ambient concentrations. Weekly monitoring of NO_3-N and PO_4-P showed the average concentrations in the sea water of Group 3 to be 2.01 and 1.02 ppm, respectively, while the concentrations for untreated Group 1 were 0.29 and 0.09 ppm. The addition of the nutrients to Group 3, therefore, maintained NO_3-N concentrations about 7 times greater than ambient levels and PO_4-P concentrations about 11.5 times greater than ambient levels.

The animals in Groups 4 and 5 were treated 3 times/wk with a solution of the chemotherapeutic, malachite green to a final concentration of 1 ppm. Malachite green has been shown to be useful in reducing the effects of epibiotic fouling on eggs and larvae of the American lobster, *Homarus americanus* (Fisher et al. 1976). Similarly, Group 7 was treated with 1590 U penicillin-G and 1 ppm streptomycin sulfate 3 times/wk. A 1-ppm concentration of streptomycin has been reported to be effective in controlling the filamentous bacterium *Leucothrix mucor* (S. Serfling et al. unpublished data). The treatment periods were conducted with the water supply shut off for ½ h. The chemicals were diluted when normal flow rates were resumed. A dark condition was provided for Groups 5 and 6 by covering the tanks with black polypropylene sheets to restrict the growth of photosynthetic microorganisms.

Small clusters of eggs were removed from the egg masses with curved forceps (1–2 cm from the posterior end of the abdomen, anteriorly directed along the midventral line) 7, 14, and 21 days after the eggs were extruded. Two setae from each cluster were placed in a drop of sea water on a microscope slide and were gently covered with a glass coverslip. Using a compound microscope (125×), the 1st 50 eggs on the distal end of each seta were examined for mortalities and the depths of mortality occurrence in the egg masses were recorded in millimeters from the distal egg. In addition, dead eggs were categorized as having either intact egg membranes (Type I mortalities) suggesting no mechanical damage to the egg, or obviously torn egg membranes (Type II mortalities) where mechanical damage could be inferred.

The eggs were examined under higher magnification (312×) for the presence of filamentous fouling. Filaments >0.034 mm long (easily detectable length at this magnification) occurring on the circumferences of 10 live eggs from each seta were counted. For this examination, a live egg was randomly selected at each millimeter depth from 0 to 9 mm inclusive. Random examinations were made of newly extruded eggs which determined that no filaments or mortalities were initially present.

The filament number and mortality data were averaged and tested for significance by analysis of variance (ANOVA) and the t statistic for the means of two samples. Wherever groups were not significantly different at the 0.05 level, they were grouped for presentation.

Results — Group 3 (nutrient enriched) showed the highest number of filaments per sample and Groups 4 and 5 (malachite green treated, light and dark) had the lowest (Fig. 1). Antibiotic-treated Group 7 shows a continuous increase in the number of filaments throughout the 21 days. The t statistic for two means applied to the filament data showed that Group 1 was not significantly different than Groups 2 and 6 and similarly that Group 4 was not significantly different than Group 5 at the 0.05 level.

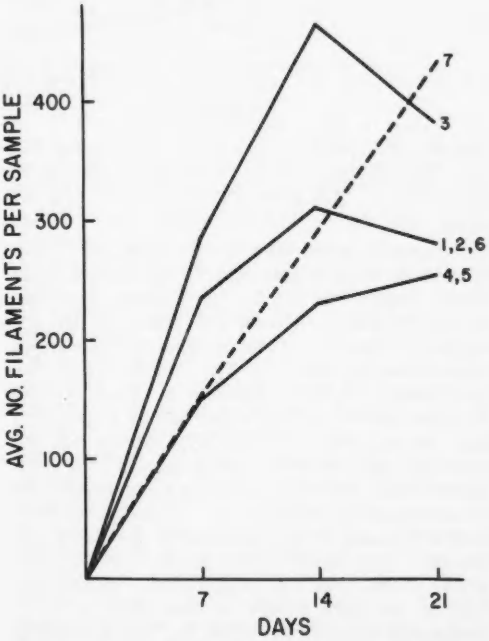

FIG. 1. Average number of filaments per sample for each examination date. Pooled Groups 1, 2, and 6 and Groups 4 and 5 were statistically similar. Statistics were as follows: Day 7 — ANOVA: df = 6, 35; $F = 4.49$; $0.005 > P > 0.001$; SE 34.0, range 28.9–43.6. Day 14 — ANOVA: df = 6, 37; $F = 4.24$; $0.005 > P > 0.001$; SE 36.1, range 12.5–54.5. Day 21 — ANOVA: df = 6, 28; $F = 3.57$; $0.01 > P > 0.005$; SE 42.6, range 23.7–72.2.

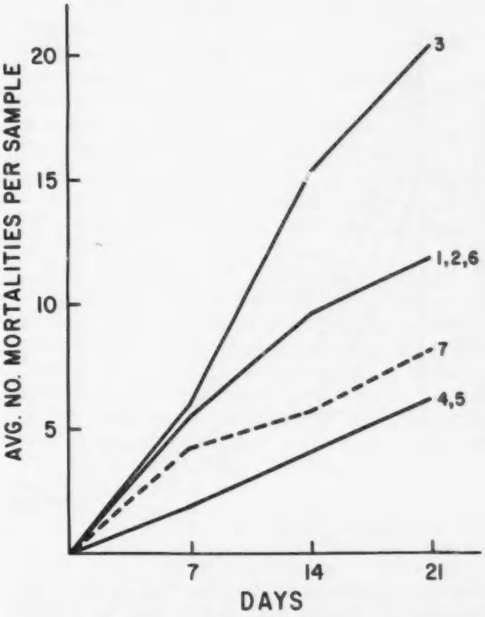

FIG. 2. Average number of mortalities per sample for each examination date. Pooled Groups 1, 2, and 6 and Groups 4 and 5 were statistically similar. Statistics were as follows: Day 7 — ANOVA: df = 6, 35; $F = 1.98$; $0.1 > P > 0.05$; SE 1.2, range 0.5–2.0. Day 14 — ANOVA: df = 6, 37; $F = 6.85$; $P < 0.001$; SE 1.4, range 0.8–2.5. Day 21 — ANOVA: df = 6, 28; $F = 8.84$; $P < 0.001$; SE 1.6, range 0.7–2.3.

The highest mortalities were found in Group 3 and the lowest in Groups 4 and 5 (Fig. 2). Mortalities for each group increased with time. The day 7 examination showed relatively little difference between the groups (analysis of variance $F = 1.98$), while day 14 and 21 examinations showed increasing differences (analysis of variance $F = 6.85$ and 8.84, respectively). A t statistic for the means of two samples showed that the mortalities from Groups 1, 2, and 6 were not significantly different and mortalities from Groups 4 and 5 were not significantly different at the 0.05 level.

Utilizing the pooled data, filament numbers (Fig. 3) and mortalities (Fig. 4) describe an exponential decline with increasing depth into the egg mass. The curves for Group 7 (Fig. 3 and 4) show that there were more filaments and mortalities on the peripheries of the egg masses than there were in the control Groups 1, 2, and 6, yet they declined at a greater rate with increased depth. Correlation coefficients for the curves in Fig. 3 are greater than 0.99 and those in Fig. 4 are greater than 0.88.

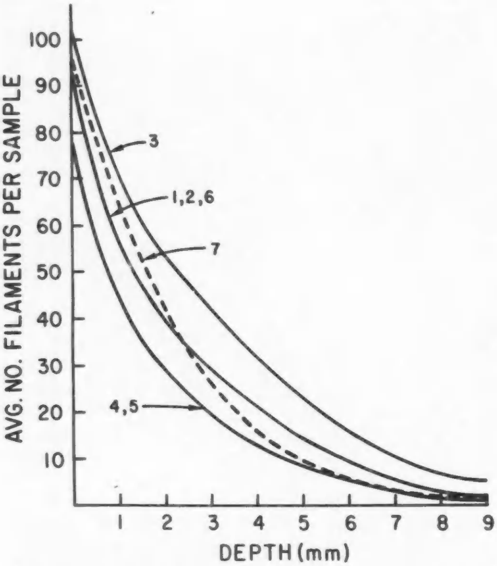

FIG. 3. Occurrence of filaments from 0 to 9 mm into the egg masses. Standard errors for all samples taken at each depth averaged 3.7 and ranged from 0.7 to 10.3.

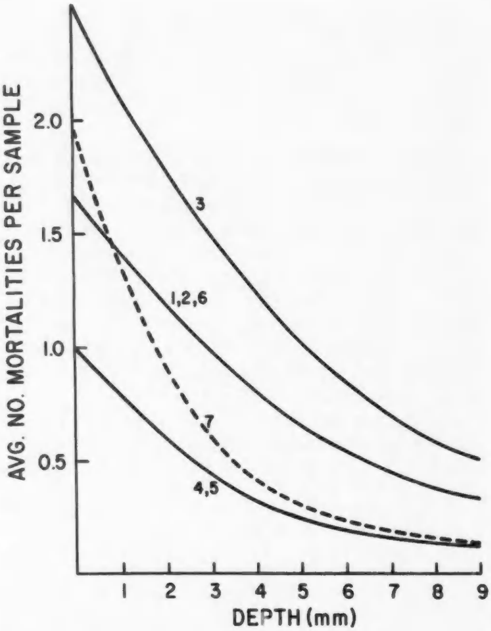

FIG. 4. Occurrence mortalities from 0 to 9 mm into the egg masses. Standard errors for all samples at each depth averaged 0.21 and ranged from 0.05 to 0.42.

Dead eggs with intact membranes (Type I) accounted for the majority of the mortalities and decreased exponentially ($r = 0.97$) with increased depth into the egg masses (Fig. 5). Dead eggs with obviously torn membranes (Type II) were fewer and occurred linearly ($r = 0.91$) along the egg-bearing setae.

Nearly all of the filaments observed in this study were cyanophytes, predominantly similar to the Oscillatoraceae. *Anabaena*-like filaments and thin, nonsegmental filaments were found in greater proportions in the dark conditions (Groups 5 and 6) than in the light conditions.

Discussion — The results of this study provide two indirect means to support the suggestion that microbial fouling can cause mortalities in egg masses of *C. magister*. The first relies on the manipulation of microorganisms with the use of nutrient enrichment and chemotherapy (malachite green). The respective higher (Group 3) and lower (Groups 4 and 5) levels of filamentous fouling due to these treatments (Fig. 1) concurred with higher and lower levels of egg mortalities (Fig. 2). The second indirect support derives from the similar decline of both filaments (Fig. 3) and mortalities (Fig. 4) with increased depth into the egg masses.

Although filament numbers appear to be highly associated with mortalities, the results of Group 7 indicate that nonfilamentous fouling is more likely responsible for the mortalities. The filaments in Group 7 were not restricted by the antibiotic treatment (Fig. 1), but the mortalities were reduced (Fig. 2). This suggests that the antibiotics had a bacteria-static effect on the nonfilamentous fouling, which allowed increased egg survival even though filamentous fouling steadily increased. It is consistent that the antibiotic-insensitive microorganisms (filaments) were predominantly algal. The antibiotic-sensitive microorganisms would presumably be free-living bacteria and bacterial filaments too short (<0.034 mm) or too thin for enumeration. Filamentous fouling has been shown in this study to be a useful and measurable index, but the mortalities can be substantially attributed to the effects of nonfilamentous fouling.

Malachite green was capable of restricting both filamentous and nonfilamentous microorganisms causing a reduction in filament numbers and mortalities. The addition of nutrients enhanced both forms of epibiotic fouling causing an increase in filament numbers and mortalities. The dark condition did not alter either the filament

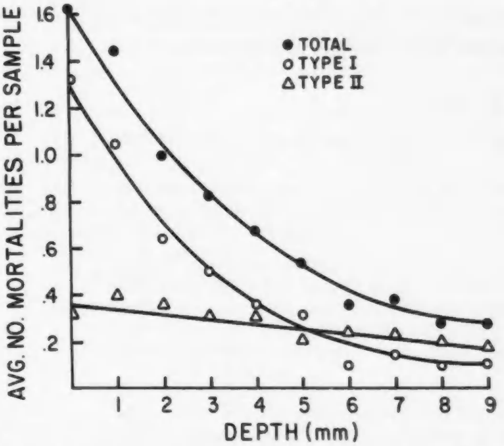

FIG. 5. Occurrence of intact membrane mortalities (Type I) and torn membrane mortalities (Type II) from 0 to 9 mm into the egg masses. Curves represent averages of all groups on all three examination dates.

numbers or the mortalities, but did cause a shift in the type of filament observed.

The distinction between Type I (intact egg membrane) and Type II (torn egg membrane) mortalities was made to use observational data to gain insight into causal factors. Type I mortalities were highly correlated with filamentous fouling while Type II mortalities were not as prevalent and were not exponentially distributed (Fig. 5). Type I mortalities were possibly a result of microbial fouling and Type II a result of the egg predator *Carcinonemertes epialti* (Kuris 1973) which also occurred in the egg masses. However, the nemertean worm is purportedly capable of penetrating the egg to feed on yolk material without immediately tearing the egg membrane (D. E. Wickham personal communication). It is also possible that Type I mortalities, regardless of the cause, can degenerate into Type II mortalities. This latter possibility is consistent with the observation (unpublished data) that the majority of mortalities found in late developmental stages (80–90 days) are Type II. Although the data presented in Fig. 5 may suggest distinct causes of mortality, the lack of further information makes this uncertain.

Extrapolation of the mortalities from nutrient enriched Group 3 (Fig. 2) would indicate approximately 90% egg mortalities after 3 mo of extruded egg development. A recent field sample from the Drakes Bay region (35 km north of the mouth of San Francisco Bay) showed 95% mortality at a similar developmental stage (unpublished data). Although this was an exceptional case, the number of mortalities observed in the laboratory under artificial conditions can feasibly occur in nature.

The data presented in this study support the initial implications of the field work (Fisher and Wickham 1976). Direct causality, however, has not been shown and the mechanics of death have been assumed. Filamentous fouling of live eggs precludes the possibility that fouling occurs merely as a result of mortality, but factors not considered in this study may have a relevant bearing on the observed mortalities.

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FISHER, W. S., E. H. NILSON, L. F. FOLLETT, AND R. A. SHLESER. 1976. Hatching and rearing lobster larvae (*Homarus americanus*) in a disease situation. Aquaculture 7: 75–80.

FISHER, W. S., AND D. E. WICKHAM. 1976. Mortalities and epibiotic fouling of eggs from wild populations of the Dungeness crab, *Cancer magister*. U.S. Fish Wildl. Serv. Fish. Bull. 74: 201–207.

KURIS, A. 1973. Population interactions between a shore crab and two symbionts. Ph.D. Thesis. Univ. California, Berkeley, Calif.

NILSON, E. H., W. S. FISHER, AND R. A. SHLESER. 1975. Filamentous infestations observed on eggs and larvae of cultured crustaceans. Proc. 6th Annu. Workshop World Mariculture Soc., Seattle, Wash. 1975. (In press)

ORCUTT, H. G., R. N. TASTO, AND P. W. WILD. 1975. Dungeness crab research program. Calif. Dep. Fish Game Mar. Resour. Adm. Rep. 75-8: 35 p.

Acute Toxicity of Residual Chlorine in Wastewater to Coho Salmon (*Oncorhynchus kisutch*) and Some Resultant Hematologic Changes

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BUCKLEY, J. A. 1976. Acute toxicity of residual chlorine in wastewater to coho salmon (*Oncorhynchus kisutch*) and some resultant hematologic changes. J. Fish. Res. Board Can. 33: 2854–2856.

Juvenile coho salmon (*Oncorhynchus kisutch*) were exposed to treated wastewater containing lethal levels of total residual chlorine (TRCl$_2$) with seawater diluent under continuous flow conditions. The 96-h TL50 was 0.07 mg/liter TRCl$_2$ (4% effluent). Exposure resulted in a slight reduction (significant $P = .05$) in hemoglobin, a slight increase (significant $P = .05$) in methemoglobin, and a temporary decrease (not significant $P = .05$) in percentage of circulating immature erythrocytes.

BUCKLEY, J. A. 1976. Acute toxicity of residual chlorine in wastewater to coho salmon (*Oncorhynchus kisutch*) and some resultant hematologic changes. J. Fish. Res. Board Can. 33: 2854–2856.

Nous avons exposé de jeunes saumons coho (*Oncorhynchus kisutch*) à des eaux usées traitées contenant des niveaux létaux de chlore résiduaire total (Cl$_2$RT), utilisant l'eau de mer comme diluant dans des conditions de débit continu. La LT50 après 96 h est de 0.07 mg/litre de Cl$_2$RT (4% d'effluent). L'exposition entraîne une légère diminution (significative à $P = .05$) de l'hémoglobine, une légère augmentation (significative à $P = .05$) de la méthémoglogine et une diminution temporaire (non significative à $P = .05$) du pourcentage d'érythrocytes immatures en circulation.

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THE combination of free residual chlorine with the ammonia in wastewater results in the formation of combined residual chlorine (chloramines). Free and combined residual chlorine are effective disinfection agents that are toxic to many organisms in receiving waters. Compared with free residual chlorine under laboratory conditions, combined residual chlorine is reported to be slightly less toxic (Merkens 1958; Rosenberger 1971) but is more persistent in receiving waters. Information describing the toxicity of residual chlorine in sea water is limited (Brungs 1973). Holland et al. (1960) reported a "critical level" of <0.1 mg/liter residual chlorine for coho salmon (*Oncorhynchus kisutch*) in sea water; mortality was 100% in 72 h. Stober and Hansen (1974) defined a lethal threshold concentration of 0.045 mg/liter residual chlorine for juvenile chinook (*O. tshawytscha*) and pink (*O. gorbuscha*) salmon in sea water following a 2-h exposure to fluctuating concentrations. Roberts et al. (1975) reported 96-h TL50's (tolerance limit to 50% of the test fish) of 0.27–0.037 mg/liter residual chlorine for three species of estuarine fish.

The present investigation was conducted at the Municipality of Metropolitan Seattle's West Point Treatment Plant, located north of Elliott Bay on Puget Sound. Tests at this laboratory have established that the toxicity of the treatment plant effluent is determined principally by the total residual chlorine (TRCl$_2$) content. In addition, static toxicity tests with coho salmon have defined a 96-h TL50 of 0.1 mg/liter TRCl$_2$ (Buckley and Matsuda 1972). Measurement of the effect of TRCl$_2$ on hemoglobin was prompted by the report of Eaton et al. (1973) of formation of methemoglobin in human and fish erythrocytes exposed to combined residual chlorine. This study provides information on some of the hematologic effects of TRCl$_2$ and defines the 96-h TL50 under continuous flow conditions.

Materials and methods — Hatchery-reared yearling coho salmon (average wet weight 25 g) were divided into 10 groups of 90 fish each and acclimated to sea water for 2 wk. Testing consisted of a 96-h exposure of duplicate groups to a continuous flow of either 0% (control), 3, 6, 9, or 12% effluent (seawater diluent) in 400-liter, circular fiberglass tanks.

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Table 1. The hematology and mortality of juvenile coho salmon (*Oncorhynchus kisutch*) exposed to lethal levels of total residual chlorine in treated wastewater (seawater diluent).

Effluent (%)	0[a]	3	6	9	12
$TRCl_2$ *(mg/liter)*					
Avg[b]	0	.05	.10	.15	.20
SD	0	.03	.06	.10	.13
O-h exposure					
Avg total Hb *(g/100 ml)*	6.6[c]				
Avg MHb *(g/100 ml)*	.28[c]				
Avg MHb % of total Hb	4.2[c]				
40-H exposure					
Avg total Hb *(g/100 ml)*	6.6	6.5	5.8[d]	6.0[d]	
Avg MHb *(g/100 ml)*	.22	.67[d]	.44[d]	.66[d]	
Avg MHb % of total Hb	3.3	10.3[d]	7.6[d]	11.0[d]	
Avg % immature erythrocyte	3.2	2.2	1.6	1.1	
96-h exposure					
Avg total Hb *(g/100 ml)*	6.4	5.9			
Avg MHb *(g/100 ml)*	.27	.54[d]			
Avg MHb % of total Hb	4.2	9.2[d]			
Avg % immature erythrocyte	2.6	5.9			
Survival (%)					
24 h	$100\frac{(172)}{(172)}$	$100\frac{(172)}{(172)}$	$89\frac{(153)}{(172)}$	$85\frac{(147)}{(172)}$	$0\frac{(\ 0\ )}{(172)}$
48 h	$100\frac{(162)}{(162)}$	$100\frac{(162)}{(162)}$	$25\frac{(\ 41)}{(162)}$	$1\frac{(\ 1\ )}{(162)}$	
96 h	$100\frac{(162)}{(162)}$	$86\frac{(140)}{(162)}$	$0\frac{(\ 0\ )}{(162)}$	$0\frac{(\ 0\ )}{(162)}$	

[a] Control.
[b] Hourly.
[c] Composite sample from all groups.
[d] Significant difference $P = .05$ control vs. percent effluent.

Blood was taken from the severed caudal peduncle from a composite sample of 20 fish at 0 h and from 10 fish collected at random from each test group of 40 and 96 h. Hemoglobin (Hb) and methemoglobin (MHb) were measured by the method of Lukeman (1959) modified by filtration of the hemolysate. Giemsa-stained smears of peripheral blood were examined for determination of condition of erythrocytes. The percentage of immature erythrocytes was estimated by examination of 500 erythrocytes from each smear. Significant differences in blood measurements were determined according to the nonparametric test of Lord (1947). Mortality was recorded periodically.

Water quality factors, except ammonia (Saxton and Zadorojny 1973), were analyzed according to Standard Methods (APHA et al. 1971). Total residual chlorine ($TRCl_2$) concentrations in the tanks were estimated from values on a continuous effluent monitor and were checked by analysis of grab samples by the amperometric titration procedure with a detection limit of 0.02 mg/liter. Un-ionized ammonia (corrected to activity) and nitrite levels in the tanks were both <0.02 mg/liter. Maximum and minimum tank temperatures (14.1 and 10.1 C, respectively) approximated the temperature regime of ambient sea water. Dissolved oxygen was consistently above 7.5 mg/liter in all tanks. Differences in salinity between control (28‰) and test tanks ranged from <1 to 3‰ at the highest dilution.

Results and discussion — The 96-h TL50 was estimated to be 0.07 mg/liter $TRCl_2$ (4% effluent) by the graphical interpolation method (APHA et al. 1971).

Exposure to lethal concentrations of $TRCl_2$ resulted in a slight reduction (significant $P = 0.05$) in Hb, a slight increase (significant $P = 0.05$) in MHb, and a corresponding increase (significant $P = 0.05$) in the percent MHb of total Hb at both 40 and 96 h (Table 1). The MHb levels shown in Table 1 are lower than the 30% MHb of total Hb in fathead minnows (*Pimephales promelas*) reported by Grothe and Eaton (1975). The difference may be due largely to the much higher concentration of residual chlorine (1.5 mg/liter) used in their tests.

After 40 h of exposure, the average percentages of immature erythrocytes were slightly less (not significant $P = 0.05$) than control values and were inversely proportional to the amount of $TRCl_2$ in the test solution. Immature erythrocytes were mostly orthochromatophil normoblasts (nomenclature of Wintrobe 1946) at that time. After exposure for 96 h, hemopoietic tissue was apparently stimulated and the previous trend was reversed since the percentages of circulating immature erythrocytes in the test fish were slightly elevated above (not significant $P = 0.05$) those of the control fish. In addition to orthochromatophils, polychromatophil normoblasts were in circulation at that time. One fish additionally contained basophilic normoblasts.

In a previous study, fish exposed to sublethal levels of $TRCl_2$ showed a significant increase in the percentage of circulating immature erythrocytes after 1 wk (Buckley et al. 1976). The percentages of immature erythrocytes in the blood from moribund fish showed an inconsistent relationship with blood from similarly tested fish not showing morbidity.

Fish responded to lethal levels of $TRCl_2$ with erratic body and respiratory movements, higher position in the tanks, loss of fright response, lethargy of movement, and eventual loss of equilibrium.

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AMERICAN PUBLIC HEALTH ASSOCIATION, AMERICAN WATER WORKS ASSOCIATION, AND WATER POLLUTION CONTROL FEDERATION. 1971. Standard methods for the examination of water and waste water. New York, N.Y. 874 p.

BRUNGS, W. A. 1973. Effects of residual chlorine on aquatic life. J. Water Pollut. Control Fed. 45: 2180–2193.

BUCKLEY, J. A., AND R. I. MATSUDA. 1972. Toxicity of West Point treatment plant effluent to coho salmon (*Oncorhynchus kisutch*). Munic. Metrop. Seattle, Seattle, Wash. 12 p.

BUCKLEY, J. A., C. M. WHITMORE, AND R. I. MATSUDA. 1976. Changes in blood chemistry and blood cell morphology in coho salmon (*Oncorhynchus kisutch*) following exposure to sublethal levels of total residual chlorine in municipal wastewater. J. Fish. Res. Board Can. 33: 776–782.

EATON, J. W., C. F. KOLPIN, AND H. S. SWOFFORD. 1973. Chlorinated urban water: a cause of dialysis-induced hemolytic anemia. Science 181: 463–464.

GROTHE, D. R., AND J. W. EATON. 1975. Chlorine-induced mortality in fish. Trans. Am. Fish. Soc. 104: 800–802.

HOLLAND, G. A., J. E. LASATER, E. D. NEUMAN, AND W. E. ELDRIDGE. 1960. Toxic effects of organic and inorganic pollutants on young salmon and trout. Wash. State Dep. Fish. Olympia, Wash. 264 p.

LORD, E. 1947. The use of range in place of standard deviation in the *t*-test. Biometrika 34: 41–67.

LUKEMAN, J. M. [ed.] 1959. Clinical chemistry procedure manual. 4th U.S. Army Med. Lab., Fort Sam Houston, Tex. p. 141–144.

MERKENS, J. C. 1958. Studies on the toxicity of chlorine and chloramines to the rainbow trout. Water Waste Treat. 7: 150–151.

ROBERTS, M. H. JR., R. J. DIAZ, M. E. BENDER, AND R. J. HUGGETT. 1975. Acute toxicity of chlorine to selected estuarine species. J. Fish. Res. Board Can. 32: 2525–2528.

ROSENBERGER, D. R. 1971. The calculation of acute toxicity of free chlorine and chloramines to coho salmon by multiple regression analysis. M.Sc. Thesis. Michigan State Univ., East Lansing, Mich. 62 p.

SAXTON, S., AND C. ZADOROJNY. 1973. Determination of ammonia by the phenolhypochlorite method. J. Water Pollut. Control Fed. 45: 905–912.

STOBER, Q. J., AND C. H. HANSON. 1974. Toxicity of chlorine and heat to pink (*Oncorhynchus gorbuscha*) and chinook salmon (*O. tshawytscha*). Trans. Am. Fish. Soc. 103: 569–576.

WINTROBE, M. M. 1946. Clinical hematology. Lea and Febiger, Philadelphia, Pa. 862 p.

Inert Particle Agglutination Tests for Detection of Antibody to Enteric Redmouth Bacterium[1]

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HANSEN, C. B., AND A. J. LINGG. 1976. Inert particle agglutination tests for detection of antibody to enteric redmouth bacterium. J. Fish. Res. Board Can. 33: 2857–2860.

Carriers (charcoal particles and latex balls) were sensitized with antigen extracts of enteric redmouth (ERM) bacterium and reacted with serum samples from immunized rabbits and hatchery trout populations. Specificity of the test was evaluated using rabbit antisera prepared against several bacterial fish pathogens. The sensitivity of the test was compared with passive hemagglutination, tube agglutination, and slide agglutination procedures. Preliminary hatchery trials determined the practicality of using these tests under field conditions. The tests were shown to be rapid, reliable, sensitive, and specific serological tools. They would be suitable as supplements to recommended isolation methods in large-scale fish disease inspection and certification programs.

HANSEN, C. B., AND A. J. LINGG. 1976. Inert particle agglutination tests for detection of antibody to enteric redmouth bacterium. J. Fish. Res. Board Can. 33: 2857–2860.

Nous avons sensibilisé des porteurs (particules de charbon et boules de latex) avec des extraits d'antigène de la bactérie de la bouche rouge entérique (ERM) et les avons fait réagir avec des échantillons de sérum de lapin immunisés et de populations de truites d'élevage. Nous avons évalué la spécificité de l'essai en utilisant des antisérums de lapin préparés contre plusieurs bactéries pathogènes de poisson. Nous avons comparé la sensibilité de l'essai par des protocoles d'agglutination passive, d'agglutination en éprouvette et d'agglutination sur lamelle. Des essais préliminaires en pisciculture ont servi à déterminer la possibilité d'employer ces tests sur le terrain. Les essais se sont montrés des outils sérologiques rapides, fiables, sensibles et spécifiques. Ils conviendraient comme suppléments aux méthodes d'isolation recommandés dans les programmes d'inspection et de certification à grande échelle de maladies des poissons.

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THE traffic in fish and fish products, now practiced by both the private and public sectors of the fish-farming industry, has resulted in the trans- and intercontinental spread of a wide array of fish pathogens. Inspection and certification programs have been proposed and developed (by a number of international, national, and regional agencies) as a means of restricting the further spread of serious fish diseases (Canadian Committee on Fish Diseases 1972; Tunison 1969; Wolf 1970; Zenny 1969).

The recommended procedures for use in inspection programs are classical isolation and identification methods (Gillespie et al. 1974). Two to 10 or more days may be required for positive identification of bacterial and viral pathogens. Furthermore, clinically healthy carrier fish, infected with low numbers of pathogens, can remain undetected (Pillay 1972; Sanders 1974). These carrier fish can cause serious epizootics when introduced into previously unexposed wild or hatchery fish stocks.

An alternative to current diagnostic methods would be rapid, inexpensive serological tests to monitor for past·exposure to certifiable pathogens. A population of fish indicating past exposure could be carriers of those pathogens. A serological or similar test should be used as a supplement to the classical isolation and identification procedures. Combination of these tools would provide historical perspective on the health status of a fish population. In addition, the chances of carriers in the population remaining undetected would be greatly reduced.

The purpose of this study was to develop an inert particle agglutination (IPA) assay as an

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adjunct procedure for use in fish disease inspection and certification programs. IPA tests have found widespread application in human and veterinary medicine (Dietz et al. 1967; Erwa et al. 1969; Gordon and Lapa 1971; Klein et al. 1966; Nakamizo and Takahashi 1965). Enteric redmouth (ERM) bacterium (Ross et al. 1966), which has been responsible for significant salmonid mortalities in the western U.S., was chosen as an experimental model.

Materials and methods — Bacterial cultures — Cultures of ERM bacterium, *Aeromonas liquefaciens, A. salmonicida, Pseudomonas fluorescens*, and *Vibrio anguillarum*, were routinely cultured on Trypticase® Soy Agar (TSA). Stock slant cultures were stored at 4 C and transferred at monthly intervals. As required, working cultures were derived from the stock slants by streak isolation on TSA plates and incubated at 25 C.

Bacterial isolates used for antigen preparation were checked for purity by streak plating and inoculated into 500-ml volumes of Trypticase® Soy Broth. Broth cultures were incubated at 20 C with continuous agitation for 48 h, rechecked for purity, and harvested by centrifugation. All cell preparations were then washed twice in phosphate buffered saline.

Buffer preparation — One tenth molar phosphate buffered saline (PBS), pH 7.0, was prepared by the method of Campbell et al. (1970).

Antigen preparation — A boiled aqueous extract (BAE) antigen of the ERM bacterium was prepared by suspending wet packed cells in double distilled water to a final concentration of 5% (wt/vol) and heating the suspension in a steamer at 100 C for 60 min. Cells were pelleted by centrifugation and discarded. The supernatant antigen preparation was lyophilized, resuspended in PBS, and stored at −70 C.

A trichloroacetic acid (TCA) extract antigen of the ERM bacterium was prepared by the method of Freeman et al. (1940). The antigen preparation was lyophilized, resuspended in PBS, and stored at −70 C.

Bacterin preparations of ERM bacterium, *A. liquefaciens, A. salmonicida, P. fluorescens*, and *V. anguillarum*, were made by the method of Campbell et al. (1970). The cells were adjusted to a final concentration of 1.2×10^9 cells/ml using McFarland nephelometry standards (Kolmer et al. 1951).

Antisera preparation and collection — New Zealand white rabbits, 5 kg average wt of either sex (Laboratory Associates, Kirkland, Washington), were used for the production of hyperimmune antisera. Bacterin preparations emulsified with an equal volume of Freund's complete adjuvant were injected subcutaneously under the left front and right rear foot pads (0.5 ml/foot pad). After 14 days, the opposite foot pads were injected. The immunization schedule was completed by intravenous injections of 0.5 ml bacterin without adjuvant into marginal ear veins at weekly intervals, until peak titers were obtained. Blood was collected by cardiac puncture and the serum separated using standard methodology (Campbell et al. 1970). Normal rabbit serum (NRS) used for controls was purchased commercially (Difco). All sera were stored at −70 C.

Field trials — To evaluate the potential value of IPA assays in actual field situations, 190 individual fish serum samples from three hatcheries were collected and serologically examined. Following anesthetization, the caudal peduncle was severed and blood collected in heparinized microhematocrit tubes. The filled tubes were centrifuged, broken at the blood plasma interface, diluted 1:10 in PBS–BSA, and frozen at −70 C.

Serological procedures — IPA tests were performed using a modification of the method of Gordon and Lapa (1971). One volume of stock latex balls (0.81 μm diam (Difco) or charcoal particles (2.5 mg/ml) (Hynson, Westcott, and Dunning, Inc., Baltimore, Md.) was centrifuged at 5000 × *g* for 10 min at 4 C and resuspended in 1 vol of PBS. One volume of antigen (diluted in PBS) was added. The mixture was thoroughly agitated and then incubated at 37 C for 1 h. Following incubation, 1 vol of 0.85% NaCl containing 1% bovine serum albumin (BSA) was added to prevent nonspecific agglutination. The mixture was incubated at 25 C for 10 min and then centrifuged as above. The pellet was washed once, resuspended in 3 vol of PBS containing of 0.1% BSA (PBS–BSA), and stored at 4 C. Antiserum was diluted in twofold increments in PBS–BSA. The agglutination test was performed by adding 25 μl of sensitized particles and 50 μl antibody dilution to individual wells on ringed, 18 × 20 slides (latex agglutination) or 10-spot teardrop Brewer diagnostic cards (Hynson, Westcott, and Dunning, Inc.) (charcoal agglutination), mixing with an applicator stick, and rotating at 100 rpm for 10 min at 20 C. Reactions were read immediately. Nonsensitized particles and NRS were used as controls for nonspecific agglutination.

Tube and slide agglutination tests followed the methodology of Campbell et al. (1970). Passive hemagglutination was performed with the microtiter modification described by Conrath (1972).

Results and discussion — To maximize the sensitivity of IPA tests, the conditions of carrier particle sensitization with antigen must be carefully controlled. If antigen is either too concentrated or too diffuse on the particle surface, the sensitivity of the agglutination test is decreased. Optimum sensitization was determined by varying buffer pH and holding antigen concentration constant and vice versa in the sensitization procedure, while holding ionic buffer strength, temperature, and incubation time constant. The titer of the sensitized preparations was determined using the latex particle agglutination test and the charcoal agglutination test using both the BAE and TCA antigen.

In the latex agglutination test the BAE antigen gave higher titers than the TCA antigen, the antigen concentration reaching an optimum and titer decreasing at either higher or lower concentrations. In the latex agglutination test, the BAE antigen was optimally bound between a pH of 6 and 8 whereas the TCA antigen was optimally bound between a pH of 7 and 12.

Using the BAE a two-fold higher titer was achieved in the charcoal agglutination test than in the latex test. As in the latex test the BAE antigen was superior to the TCA antigen in achieving optimum sensitization. With both antigens sensitization optimum concentrations were reached and titers decreased at antigen concentrations higher or lower than the optimum. Binding of both antigens was pH independent between pH 5 and 12 in the charcoal agglutination test implying that binding was adsorptive rather than ionic with the charcoal system. No titers were observed with nonsensitized latex or charcoal particles or with normal rabbit serum in any of the tests.

Determination of the specificity of IPA tests — Antisera against ERM bacterium, *A. liquefaciens*, *A. salmonicida*, *P. fluorescens*, and *V. anguillarum*, were reacted with carrier particles sensitized with ERM–BAE and ERM–TCA antigens. The bacteria chosen as controls all produce clinical symptons similar to ERM (i.e. hemorrhagic septicemia) and could conceivably cause problems in accurate disease diagnosis.

Carrier particles sensitized with BAE antigen reacted only with anti-ERM antiserum. Neither cross-reactions with the other hyperimmune antisera nor reactions with unsensitized particles were detected.

Determination of the sensitivity of IPA tests — IPA, tube and slide agglutination, and passive hemagglutination (PHA) techniques were compared to determine relative sensitivity, using ERM antigen preparations and hyperimmune anti-ERM antiserum. The results of this study are presented in Table 1.

TABLE 1. Comparison of sensitivity of serological tests using ERM antigens and homologous hyperimmune antiserum.

Test	ERM antigen	Titer
Charcoal particle agglutination	TCA	512[a]
	BAE	1024
Latex agglutination	TCA	256
	BAE	1024
Tube agglutination	Cell	256
Slide agglutination	Cell	64
Passive hemagglutination	TCA	128
	BAE	4096
	BAE	1024[b]

[a] Values presented as reciprocal of highest dilution showing a positive reaction.

[b] After heat-treatment of serum at 37 C for 60 min.

The titers from the charcoal particle and latex particle agglutination tests are comparable to those of the passive hemagglutination test (serum heated to prevent nonspecific agglutination) which was the most sensitive method tested. Instability of reagents and hemolytic reactions make the passive hemagglutination test difficult to use in field certification programs. The difference in sensitivity between the BAE and the TCA antigen would indicate that the BAE antigen used in the charcoal particle method would be the procedure of choice for further trials with this system.

Field trial results — Serum samples from three hatchery trout populations were analyzed for the presence of specific anti-ERM antibodies, using the IPA procedure. Charcoal particles sensitized with ERM–BAE antigen were tested with serum diluted 1:10 in PBS–BSA to eliminate possibility of nonspecific agglutination.

Hatchery A historically had few problems with ERM and reported no recent outbreaks of the disease at the time of sampling. The lack of previous exposure was reflected in the absence of positive serum titers in the 84 samples analyzed. Hatchery B had experienced past problems with ERM disease. However, at the time of sampling, no recent outbreaks had occurred. One out of the 46 samples tested had a positive anti-ERM serum titer. Hatchery C had reported an outbreak of ERM approximately 1 mo prior to sampling. Seven of the 60 serum samples analyzed were positive for anti-ERM antibodies.

The field trials described above, though limited in scope, establish the ability of IPA tests to detect past exposure to ERM bacterium. However, before these tests can be used by fish health specialists, several further developments are required. The tests will have to be proven capable of detecting past exposure to other certifiable bacterial, viral, and internal protozoan pathogens. This study and studies by other investigators cited earlier indicate that IPA tests have such a capability. Suitable antigen extracts from each of the certifiable pathogens will have to be isolated and optimally bound to carrier particles. Where a given pathogen exists in two or more serotypes, polyvalent antigen-carrier suspensions will be required. This preliminary work will then have to be followed by extensive field trials, where comparisons between the IPA tests and classical isolation and identification methods can be made.

IPA tests appear ideally suited for use in large-scale serological surveillance programs. They are rapid, reproducible, sensitive, and specific. Sensitized particle suspensions can be made in advance and are stable at refrigerator temperatures for up to a year. The serum volume is small (25 μl) and can be obtained by microhematocrit sampling. Once a serum sample is prepared, the actual test requires <10 min to complete.

IPA tests can also be developed into kit form. Particle suspensions sensitized with antigen extracts of each of the certifiable pathogens could be packaged with nonwettable ringed cards, micropipettes, buffer, and positive and negative control sera. Fish serum samples would then be run against the entire series of antigen-sensitized carrier suspensions.

Acknowledgments — The standard charcoal suspension and Brewer diagnostic cards were supplied by Dr G. F. Whatlan (Hynson, Westcott, and Dunning, Inc., Baltimore, Md.). Cultures of *A. liquefaciens*, *A. salmonicida*, *P. fluorescens*, and *V. anguillarum* were furnished by A. J. Ross (Western Fish Disease Laboratory, Seattle, Wash.).

This research was supported in part by Grant H-649 from the Idaho Agricultural Experiment Station and in part by STAR (Short Term Applied Research) funds authorized by the legislature of the State of Idaho.

CAMPBELL, D. H., J. S. GARVEY, N. E. CREMER, AND D. H. SUSSDORF. 1970. Methods in immunology. W. A. Benjamin, Inc., New York, N.Y. 454 p.

CANADIAN COMMITTEE ON FISH DISEASES. 1972. Considerations and recommendations for the control of fish diseases in Canada. Fish. Mar. Serv. Misc. Spec. Publ. 16: 52 p.

CONRATH, T. B. 1972. Handbook of microtiter procedures. Dynatech Corp., Cambridge, Mass. 475 p.

DIETZ, T. M., K. W. MAXWELL, J. L. ROBBINS, AND S. MARCUS. 1967. Use of latex particles in serology of tuberculosis. Am. Rev. Respir. Dis. 95: 638–642.

ERWA, H. H., W. R. MAXTED, AND W. D. BRIGHTON. 1969. A latex agglutination test for the measurement of antibodies to group-specific streptococcal polysaccharides. Clin. Exp. Immunol. 4: 311–321.

FREEMAN, C. C., S. W. CHALINOR, AND J. WILSON. 1940. The use of a synthetic medium in the isolation of the somatic antigens of *Bacterium typhimurium* and *Bacterium typhosum*. Biochem. J. 34: 307.

GILLESPIE, D. C., T. P. T. EVELYN, C. FRANTSI, R. M. MACKELVIE, AND N. NEUFELD. 1974. Methods for the detection of certain pathogens of salmonid fishes. Fish. Mar. Serv. Misc. Spec. Publ. 23: 19 p.

GORDON, M. A., AND E. LAPA. 1971. Charcoal particle agglutination test for detection of antibody to *Cryptococcus neoformans*: a preliminary report. Am. J. Clin. Pathol. 56: 354–359.

KLEIN, M., S. CHAEFSKY, AND M. MULLER. 1966. A rapid agglutination test for the detection of antibodies to viruses of influenza. J. Immunol. 97: 131–137.

KOLMER, J. A., E. H. SPAULDING, AND H. W. ROBINSON. 1951. Approved laboratory technic. 5th ed. Appleton-Century-Crofts, New York, N.Y. 1180 p.

NAKAMIZO, Y., AND R. TAKAHASHI. 1965. Latex agglutination test in shigellosis and salmonellosis. Am. J. Trop. Med. Hyg. 14: 783–786.

PILLAY, T. V. R. 1972. Editorial. FAO Aquacult. Bull. 4: 1–2.

ROSS, A. J., R. R. RUCKER, AND W. H. EWING. 1966. Description of a bacterium associated with redmouth disease of rainbow trout (*Salmo gairdneri*). Can. J. Microbiol. 12: 763–770.

SANDERS, J. 1974. Fish health section newsletter. May 1974. p. 2.

TUNISON, A. V. 1969. A proposed disease control plan for the fish industry. Am. Fish U.S. Trout News, Sept.–Oct. 1969. p. 8–9, 12–15.

WOLF, H. 1970. Problems associated with the operation of fish inspection programs in California, p. 36–40. *In* S. F. Snieszko [ed.] A symposium on diseases of fishes and shellfishes. Am. Fish. Soc., Washington, D.C. Spec. Publ. 5.

ZENNY, F. B. 1969. Comparative study of laws and regulations governing international traffic in live fish and fish eggs. EIFAC Tech. Pap. 10: 52 p.

Modification of the Whiteside–Williams Pattern Sampler[1]

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BRAKKE, D. F. 1976. Modification of the Whiteside–Williams pattern sampler. J. Fish. Res. Board Can. 33: 2861–2863.

The construction and operation of a modified pattern sampler for littoral microcrustaceans is described. The sampler is lightweight and inexpensive, yet produces quantitative information on populations and their spatial array. The composition of collections made by net and pattern samplers was not significantly different. The pattern sampler, however, offers several advantages.

BRAKKE, D. F. 1976. Modification of the Whiteside–Williams pattern sampler. J. Fish. Res. Board Can. 33: 2861–2863.

L'auteur décrit la construction et l'opération d'un échantillonneur modifié permettant d'étudier les modalités de répartition des micro-crustacés du littoral. Léger et peu dispendieux, l'échantillonneur fournit quand même une information quantitative sur les populations et la façon dont elles sont disposées dans l'espace. Une comparaison des échantillons recueillis au filet et à l'échantillonneur indique que leur composition n'est pas significativement différente. L'échantillonneur, cependant, offre plusieurs avantages.

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WHITESIDE and Williams (1975), elaborating on the ideas of Szlauer (1963), devised a sampler that yields quantitative information on populations of littoral microcrustaceans. It is now possible to obtain information on the population size, spatial distribution, and quantitative dynamics of aquatic invertebrates.

The Whiteside–Williams sampler, although well adapted for lake sampling, is not without drawbacks. It is expensive ($750 each) and cumbersome, requiring manipulation by a winch and a diver. The following is a description of a modified pattern sampler that is inexpensive, easy to construct, lightweight, and operable by a single individual.

Sampler design and construction — The Whiteside–Williams pattern sampler is an 8 × 8 matrix of square funnels, each 45 cm^2, with an effective sampling area of 2,767 cm^2. The modified sampler assembled according to the design in Fig. 1 captures animals over an area of 794 cm^2. In this sampler, the animals must swim upward a distance of 9.0 cm in order to become trapped in the sample bottles.

My sampler is constructed from plexiglass (6.35 mm thick), polyethylene funnels (58 mm maximum ID), and polypropylene bottles (30 ml). The stems of funnels are inserted through holes in the plexiglass and glued in place with 1,2 dichloroethane. As the stems of these funnels narrow rapidly, it is advantageous to remove the stem and insert in its place plastic tubing 4 cm long (bore size 6.35 mm). The caps do not bond well to plexiglass and hence require a high-grade contact cement. The total cost of materials necessary for the construction of two samplers is about $70.

Sampler operation and its efficiency — The sampling procedure is identical to that of Whiteside and Williams (1975). The sampler is placed over a particular substrate (aquatic vegetation, bare lake sediments, etc.) in the evening and retrieved the following morning. The animals, being most active at night, crawl or swim up the funnels and are captured in the bottles. Sampling efficiency is greater than 90% (Whiteside and Williams 1975).

I tested a pair of modified samplers in the field in the fall of 1975 (Monroe Co., Bean Blossom Lake, Indiana). They proved to be effective and durable. The samplers placed in beds of *Najas flexilis* trapped individuals of all species of chydorid Cladocera and all stages of *Camptocercus*

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[1] Contribution No. 1033, Department of Zoology, Indiana University, Bloomington, Indiana 47401, USA.

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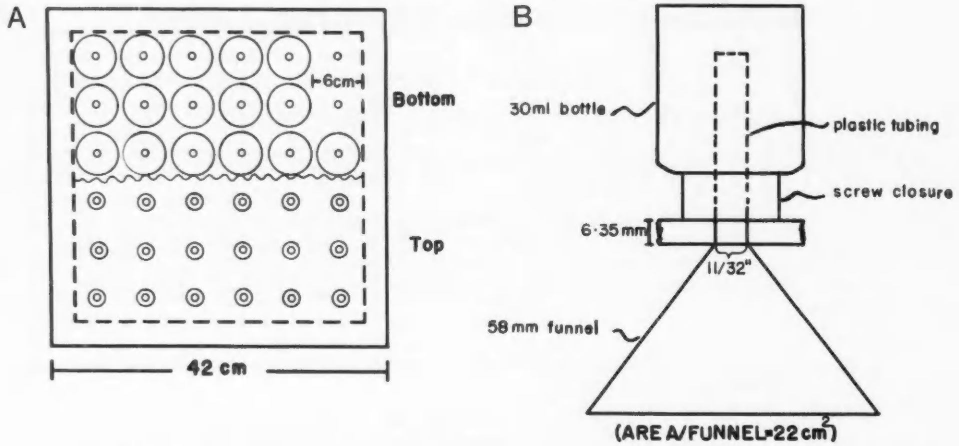

FIG. 1. Modified pattern sampler: A, entire sampler; B, individual funnel and collecting bottle.

TABLE 1. Comparison of net and pattern samples; all chydorid species, Bean Blossom Lake, Indiana. Percentages with *Graptolebris* excluded.

Species	Net count	%	Pattern count	%
Camptocerus sp. Σ individuals	190	20.47	264	17.18
parthenogenetic ♀♀	(87)	(9.38)	(159)	(10.27)
parthenogenetic ♀♀ with eggs	(27)	(2.90)	(49)	(3.17)
gamogenetic ♀♀	(3)	(0.32)	(5)	(0.32)
Σ ♀♀	(180)	(19.39)	(248)	(15.69)
Σ ♂♂(immature)	(10)	(1.08)	(23)	(1.49)
Pleuroxus denticulatus	115	12.39	76	4.91
Chydorus sphaericus	232	25.00	401	25.90
Alona setulosa	322	34.70	744	48.06
Alonella excisa	70	7.54	65	4.20
Graptolebris testudinaria	698		352	
Pseudochydorus globosus	2	0.22	1	0.06
Σ *N*	1629		1903	

present in the lake, the latter being a particular object of study.

Estimates of chydorid population size (all species) ranged from 20,000 to 40,000 animals/m². Although these values are somewhat lower than those for fall populations in *Chara* in Lake Itasca, Minnesota (M. C. Whiteside personal communication), the differences are probably attributable to habitat influences and not lower sampler efficiency. Both kinds of samplers produced similar population estimates during the summer of 1976 in Lake Itasca. No data are available yet from Minnesota or Indiana to indicate the range in autumnal population maxima that might be expected from one lake to another.

Large-volume net samples were collected from the littoral vegetation on several dates when the pattern samplers were set. Subsamples were counted and the results compared to data from the pattern samplers (Table 1). The two methods were highly related. A binomial expansion was applied to the rank orders of species in net and

TABLE 2. Comparison of net and pattern samples; *Camptocerus*, χ^2 2 × 2 contingency tests

			♀♀			
	Mature ♀♀	Immature ♀♀	with eggs	without eggs	♂♂	♀♀
Net sample	141	153	32	95	63	294
Pattern sample	91	65	23	67	37	156
	$\chi^2 = 0.399$		$\chi^2 = 0.010$		$\chi^2 = 0.107$	

pattern samples and the resultant probability of chance concordance was less than .001. One organism, *Graptolebris testudinaria*, was not retained as efficiently by the pattern sampler. As a result, population estimates for this species appear about 50% low.

Camptocercus individuals were examined in greater detail to show that all life stages were sampled with equal efficiency. They were measured to obtain size distributions and categorized according to reproductive condition. An analysis was made by 2 × 2 contingency tests to compare the populations collected by the net and pattern samplers (Table 2). All χ^2 values indicate no significant differences ($P > .05$) between the populations.

Pattern sampling is recommended over other sampling techniques. Results generated from pattern samples have several advantages: they are quantitative, give information on spatial array, maintain environmental variability (in spatial distributions), and tend to be more efficient in sampling species that are infrequent or mud-dwelling.

Acknowledgments — I profited from discussions of sampling techniques with Dr M. C. Whiteside, J. B. Williams, and Professor D. G. Frey. Drs Frey and T. L. Crisman kindly provided detailed comments on the manuscript. The research was supported by the Indiana Academy of Science.

SZLAUER, L. 1963. Diurnal migrations of minute invertebrates inhabiting the zone of submerged hydrophytes in a lake. Schweiz. Z. Hydrol. 25: 56–64.

WHITESIDE, M. C., AND J. B. WILLIAMS. 1975. A new sampling technique for aquatic ecologists. Verh. Int. Ver. Limnol. 19: 1534–1539.

LETTERS/LETTRES

Freon as a Solvent for Determining Fat Content of Fish

Comment

VLADO ZITKO

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A paper by Korn and Macedo (1973) recommends the use of MF freon (monofluorotrichloromethane) for the determination of fat content in fish.

A rather large quantity of this solvent is required per sample (100 ml) and the solvent is not recovered but evaporated to atmosphere in a fumehood.

The described procedure is not sound environmentally, particularly in view of the possible effects of freons on the ozone layer. Even freons in aerosol cans may be banned. Another recoverable solvent, perhaps the hexane–acetone azeotropic mixture, should be used.

Maybe the authors would like to comment and you might want to publish a notice on the environmental hazards of MF freon in the *Journal*.

KORN, S., AND D. MACEDO. 1973. Determination of fat content in fish with a nontoxic, noninflammable solvent. J. Fish. Res. Board Can. 30: 1880–1881.

Reply

SID KORN

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Dr Zitko makes the strong point of not using MF freon (monofluorotrichloromethane) as a solvent in the determination of fat content in fish due to concerns over possible effects of freon on the ozone layer in the atmosphere.

Until the question of freon's effect on the environment is settled, I feel investigators must consider the safety advantages of freon over other suggested solvents since it is relatively nontoxic and nonflammable. The hexane–acetone mixture suggested would be highly flammable. Modification of the fat extraction method to recover the freon used is possible through appropriate condenser Soxhlet apparatus and is desirable.

Editorial Comment

Both scientists have made valid points. Unfortunately the stratosphere problem with respect to freons is not resolved but is still very much a controversial one. Nevertheless, monofluorotrichloromethane is considered to be one of the most hazardous of the freons and it would be desirable if another freon solvent could be substituted for it. In any case, recovery of the freon should be attempted by anyone using the procedure for we cannot condone the unnecessary pollution of the atmosphere.

It might be noted a) that the hexane–acetone azeotropic mixture is still commonly used in laboratories as a solvent for extraction of lipids and b) that the amount of freon released in the described analytical procedure, compared to that from industry, is extremely small. — L.C.S.

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Books Received/Livres reçus
June 1976

1975 Compendium of Current Environmental Studies in Puget Sound and Northwest Estuarine Waters. *Edited by Saskia Schott and Joseph Pizzo. The Oceanographic Institute of Washington, Seattle, Wash., 1976. 434 p. $16.00.*

(A looseleaf index of 331 studies in the marine and estuarine waters of southwest Canada, Washington State marine waters, and the lower Columbia River. There are 14 major subject categories. The investigator, his affiliations, the geographical location of the project, an abstract, the supporting agencies, the project's status, and the research type are given.)

*The Proceedings of the Bras d'Or Lakes Aquaculture Conference.** *Edited by Gregory McKay. College of Cape Breton Press, Sydney, Nova Scotia, 1976. xvi + 312 p. $15.00.*

(A comprehensive report on aquaculture in Atlantic Canada and the major concerns and problems which need attention as seen by those directly affected or responsible for support.)

*Sea Turtles and the Turtle Industry of the West Indies, Florida, and the Gulf of Mexico.** *Thomas P. Rebel. University of Miami Press, Coral Gables, Fla., 1974. 250 p. $10.00.*

(A revised and expanded edition of Ingle and Smith's handbook on the biologic, oceanographic, and manmade factors affecting the green, hawksbill, loggerhead, ridleys, and leatherback turtles in tropical and subtropical oceans.)

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*To be reviewed.

COMING NEXT MONTH / À PARAÎTRE LE MOIS PROCHAIN

Development of Atlantic salmon (*Salmo salar*) eggs and alevins under varied temperature regimes
R. H. Peterson, H. C. E. Spinney, and A. Sreedharan

Age determination of an elasmobranch (*Squalus acanthias*) by X-ray spectrometry
B. C. Jones and G. H. Geen

A utility function for examining policy affecting salmon on the Skeena River **R. L. Keeney**

Morphology and some observations on the population genetics of *Anisakis simplex* larvae (Nematoda: Ascaridata) from fishes of the North Atlantic **M. Beverley-Burton, O. L. Nyman, and J. H. C. Pippy**

Tissue accumulation and enzymatic effects of hexavalent chromium in rainbow trout (*Salmo gairdneri*)
D. R. Buhler, R. M. Stokes, and R. S. Caldwell

Bicinctus-group of the genus *Cricotopus* Van der Wulp (Diptera: Chironomidae) in the Nearctic with a description of a new species **D. R. Oliver**

Correlations of fish catch and environmental factors in the Gulf of Maine
W. H. Sutcliffe Jr., K. Drinkwater, and B. S. Muir

Crayfish growth in Lake Tahoe: effects of habitat variation **R. W. Flint and C. R. Goldman**

Relative resistances of three transferrin genotypes of coho salmon (*Oncorhynchus kisutch*) and their hematological responses to bacterial kidney disease **B. K. Suzumoto, C. B. Schreck, and J. D. McIntyre**

DNA, RNA, protein, and free amino acids during ontogenesis of rainbow trout (*Salmo gairdneri*)
I. H. Zeitoun, D. E. Ullrey, W. G. Bergen, and W. T. Magee

Encounter probabilities and community structure in zooplankton: a mathematical model
J. Gerritsen and J. R. Strickler

Differing goals of salmon management on the Skeena River **R. Hilborn and C. J. Walters**

Influence of lipid pool size on bioaccumulation of the insecticide chlordane by northern redhorse suckers (*Moxostoma macrolepidotum*) **J. R. Roberts, A. S. W. deFreitas, and M. A. J. Gidney**

Rate of conversion of ^{14}C-p,p'-DDT to p,p'-DDE by brook trout (*Salvelinus fontinalis*): absence of effect of pretreatment of fish with compounds related to p,p'-DDT **R. F. Addison and M. E. Zinck**

Instar succession, vertical distribution, and interspecific competition among four species of *Chaoborus*
J. C. H. Carter and J. K. Kwik

Genetic differences in growth and survival of juvenile hatchery and wild steelhead trout, *Salmo gairdneri*
R. R. Reisenbichler and J. D. McIntyre

Notes

Morphological development of the integument of the sea lamprey, *Petromyzon marinus*
W. C. Anderson and P. J. Manion

Seasonal and developmental variation in the lethality of zinc to juvenile Atlantic salmon (*Salmo salar*)
V. Zitko and W. G. Carson

Critical swimming speeds of coho salmon (*Oncorhynchus kisutch*) fry to smolt stages in relation to salinity and temperature **G. J. Glova and J. E. McInerney**

Species diversity of littoral zone fishes along a phosphorus–production gradient in Lake Memphremagog, Quebec–Vermont **B. S. Nakashima, D. Gascon, and W. C. Leggett**

Reliability of laboratory metabolic measurements of meiofauna
W. B. Vernberg, B. C. Coull, and D. D. Jorgensen

Back-calculated fish lengths and mercury and zinc levels from recent and 100-year-old cleithrum bones from Atlantic cod (*Gadus morhua*) **J. S. Scott**

Vital statistics and migratory patterns of a potamodromous stock of smallmouth bass, *Micropterus dolomieui*
W. H. Robbins and H. R. MacCrimmon

Inconnu, *Stenodus leucichthys*, migration studies in Alaska 1961–74 **K. T. Alt**

New cell line from the marine lumpfish, *Cyclopterus lumpus* **M. F. Li and S. Clyburne**

RECENT PUBLICATIONS / RÉCENTES PUBLICATIONS

The number following each title in each series refers to the establishments listed at the end of the section.

Les nombres qui suivent les titres de chacune des séries désignent les établissements suivants à la fin de la section.

Recent Studies

Until 1970, reprints of these articles had been included in an annual volume of "Studies," composed of two parts. The final binding was the 1969 Studies, Part 2, which included No. 1355. These are no longer bound and distributed. We continue, however, to number and list all articles on investigations published in other periodicals, which henceforth may be obtained in reprint form only from the author or originating establishment.

Récentes études

Jusqu'en 1970, ces articles étaient réimprimés, puis reliés dans un volume annuel d'études, comprenant deux parties. Les études de 1969, partie 2, incluant le n° 1355, ont été les dernières à être reliées. Nous n'en faisons désormais plus la reliure ni la distribution. Nous continuons cependant de numéroter les articles et d'en établir des listes d'études sur des recherches mentionnées dans d'autres périodiques. On peut donc se les procurer, sous leur forme réimprimée seulement, de l'auteur ou du service dépositaire.

2242. KOMOURDJIAN, M. P., R. L. SAUNDERS, AND J. C. FENWICK. 1976. Evidence for the role of growth hormone as a part of a "light–pituitary axis" in growth and smoltification of Atlantic salmon (*Salmo salar*). Can. J. Zool. 54: 544–551. (6)

2243. CASTELL, J. D., AND J. F. COVEY. 1976. Dietary lipid requirements of adult lobsters, *Homarus americanus* (M.E.). J. Nutr. 106: 1159–1165. (8)

Manuscript Reports

This series includes unpublished preliminary reports and data records not intended for general distribution. They should not be referred to in publications without clearance from the issuing establishment and without clear indication of their manuscript status.

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SIBERT, J., T. J. BROWN, B. A. KASK, AND J. D. FULTON. 1976. Observations on the lower trophic levels of the Cowichan estuary, Vancouver Island, B.C. Fish. Mar. Serv. MS Rep. 1394: 25 p. (1)

CADDY, J. F., C. A. DICKSON, AND M. J. A. BUTLER. 1976. Age and growth of giant bluefin tuna (*Thunnus thynnus thynnus*) taken in Canadian waters in 1975. Fish. Mar. Serv. MS Rep. 1395: 17 p. (6)

CADDY, J. F. 1976. Projection of population fecundity of Atlantic bluefin tuna (*Thunnus thynnus thynnus*) population under specified conditions of recruitment and age-specific exploitation rates. Fish. Mar. Serv. MS Rep. 1396: 10 p. (6)

Technical Report Series

Technical Reports are research documents that are of sufficient importance to be preserved, but which for some reason are not appropriate for primary scientific publication. Inquiries concerning any particular Report should be directed to the issuing establishment.

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Les rapports techniques sont des documents de recherche qui revêtent une assez grande importance pour être conservés mais qui, pour une raison ou pour une autre, ne conviennent pas à une publication scientifique de fonds. Pour toute demande de renseignements concernant un rapport particulier, il faut s'adresser au service responsable.

604. WONG, J., E. G. BAKER, AND M. YAMAMOTO. 1976. Roe herring processing: effect of freezing and thawing rates and duration of frozen storage on "spongy" roe development. Fish. Mar. Serv. Res. Dev. Tech. Rep. 604: 7 p. (2)

605. NICHOLSON, H. F. 1976. Bibliography on the limnology and fisheries of Canadian freshwaters. Appendix A. Cumulative author index. Fish. Mar. Serv. Res. Dev. Tech. Rep. 605: 121 p. (11)

606. LEVINGS, C. D., AND A. I. MOODY. 1976. Studies of intertidal vascular plants, especially sedge (*Carex lyngbyei*) on the disrupted Squamish River delta, British Columbia. Fish. Mar. Serv. Res. Dev. Tech. Rep. 606: 57 p. (3)

607. Beamish, R. J., L. M. Blouw, and G. A. McFarlane. 1976. A fish and chemical study of 109 lakes in the Experimental Lakes Area (ELA), northwestern Ontario, with appended reports on lake whitefish ageing errors and the northwestern Ontario baitfish industry. Fish. Mar. Serv. Res. Dev. Tech. Rep. 607: 116 p. (1)

608. Hargrave, B. T., G. A. Phillips, and S. Taguchi. 1976. Sedimentation measurements in Bedford Basin, 1973–1974. Fish. Mar. Serv. Res. Dev. Tech. Rep. 608: 129 p. (7)

609. Hoskins, G. E., G. R. Bell, and T. P. T. Evelyn. 1976. The occurrence, distribution and significance of infectious diseases and of neoplasms observed in fish in the Pacific region up to the end of 1974. Fish. Mar. Serv. Res. Dev. Tech. Rep. 609: 37 p. (1)

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612. Kennedy, W. A., C. T. Shoop, W. Griffioen, and A. Solmie. 1976. The 1974 crop of salmon reared at the Pacific Biological Station experimental fishfarm. Fish. Mar. Serv. Res. Dev. Tech. Rep. 612: 19 p. (1)

613. Humphreys, R. D., and C. W. Haegele. 1976. An evaluation of herring spawn survey techniques used in British Columbia waters. Fish. Mar. Serv. Res. Dev. Tech. Rep. 613: 142 p. (1)

614. Coombes, S. B. 1976. Publications December 1, 1972–December 31, 1975, Halifax Laboratory. Fish. Mar. Serv. Res. Dev. Tech. Rep. 614: 14 p. (8)

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616. Shearer, J. A. 1976. Phytoplankton primary production in the Experimental Lakes Area using an incubator technique — 1974 data. Fish. Mar. Serv. Res. Dev. Tech. Rep. 616: 142 p. (4)

617. Foy, M. G., and S. I. C. Hsiao. 1976. Phytoplankton data from the Beaufort Sea, 1973 to 1975. Fish. Mar. Serv. Res. Dev. Tech. Rep. 617: 44 p. (5)

618. Scott, J. S. 1976. Digenetic trematode parasites and food of winter flounder (*Pseudopleuronectes americanus* (Walbaum, 1792)) from the Scotian Shelf and Gulf of St. Lawrence. Fish. Mar. Serv. Res. Dev. Tech. Rep. 618: 9 p. (6)

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The following translations have been prepared by or for the Fisheries and Marine Service, for use in its investigations. Copies are available from Translation Services, Canada Institute for Scientific and Technical Information, National Research Council of Canada, Ottawa, Ontario K1A 0S2, at a cost of 20¢ per page and a minimum charge of $2.00. Information on translations can be obtained from the Office of the Editor.

Traductions

Les traductions dont il est question ci-après ont été préparées par ou pour le Service des pêches et des sciences de la mer, à des fins de recherche. On peut en obtenir des exemplaires, à 20¢ la page et à un prix minimum de $2.00 aux Services de traduction, Institut canadien de l'information scientifique et technologique, Conseil national de recherches du Canada, Ottawa (Ontario), K1A 0S2. Le Bureau du rédacteur peut fournir des renseignements sur ces traductions.

Acker, L., A. Fincke, and E. Schulte. 1972. Removal of chlorinated insecticides from cacao butter by deodorization. Susswaren 6: 245–247. (Transl. from German by Fish. Mar. Serv. Transl. No. 3786, 1976)

Melcher, F., and E. Renner. 1976. Studies on minor fatty acids in milk fat. 1. Gas-chromatographic analysis of minor fatty acids. Milchwissenschaft 31: 70–75. (Transl. from German by Fish. Mar. Serv. Transl. Ser. No. 3787, 1976)

Dziedzic, A., B. Escudie, and A. Hellion. 1975. Present methods of analysing animal sonar signals. Ann. Telecommun. 30: 270–276. (Transl. from French by Fish. Mar. Serv. Transl. Ser. No. 3788, 1976)

Utermöhl, H. 1927. Investigation of the total plankton content of the ocean off the Canary Islands. A report on studies conducted off Puerto Orotava with professor Pütter, together with a critical assessment of some investigative techniques. Arch. Hydrobiol. 18: 521–522. (summary only) (Transl. from German by Fish. Mar. Serv. Transl. Ser. No. 3789, 1976)

Valeria, V. 1970. Bacteriological and chemical characteristics of some preserved fish products. Lucr. Stiint. Inst. Agron. Bucuresti Ser. C: 13: 469–474. (Transl. from Romanian by Fish. Mar. Serv. Tarnsl. Ser. No. 3790, 1976)

Pavel'yeva, L. G., and V. V. Vlasova. 1974. Determining the degree of fat oxidation in frozen Caspian kilka. Tr. Vses. Nauchno-Issled. Inst. Morsk. Rybn. Khoz. Okeanogr. 101: 181–187. (Transl. from Russian by Fish. Mar. Serv. Transl. Ser. No. 3791, 1976)

Grishina, N. L., D. I. Kuznetsov, and L. I. Semyonova. 1976. Extractability of lipids from fish and fish products. Vopr. Pitan. 1: 65–72. (Transl. from Russian by Fish. Mar. Serv. Transl. Ser. No. 3792, 1976)

Sinyak, K. M., M. Ya. Orgel', and V. I. Kruk. 1976. Method of preparing blood lipids for gas chromatographic analysis. Lab. Delo 1: 37–41. (Transl. from Russian by Fish. Mar. Serv. Transl. Ser. No. 3793, 1976)

Apekin, V. S. 1975. The possibility of determining the gonadotropic activity of the pituitary glands of fishes by oocyte maturation *in vitro*. Tr. Vses. Nauchno-Issled. Inst. Morsk. Rybn. Khoz. Okeanogr. 96: 48–56. (Transl. from Russian by Fish. Mar. Serv. Transl. Ser. No. 3794, 1976)

Kruger, A. 1973. Application of I^{125} for labelling young eels. Rocz. Nauk Roln. 95-H-3: 69–90. (Transl. from Polish by Fish. Mar. Serv. Transl. Ser. No. 3795, 1976)

Vladimirov, O. A., A. P. Nagurnyi, Yu. B. Oliunin, and B. A. Sleptzov-Shevlevich. 1975. Certain features of the hydrologic regime of the Denmark Strait. (August to September, 1973). Tr. Issled. Krupnomasshtabnogo vzaimodeistvia okeana i atmosfery. 321: 62–72. (Transl. from Russian by Fish. Mar. Serv. Transl. Ser. No. 3796, 1976)

Furukawa, K., H. Nii, M. Iwakiri, and T. Kubota. 1976. The fatty acid composition of the seed oil of *Lindera obtusilova* Blume. Yukagaku 25: 190–193. (Transl. from Japanese by Fish. Mar. Serv. Transl. Ser. No. 3797, 1976)

Matsui, M., T. Watanabe, and T. Kawabata. 1976. Fatty acid structures of triglycerides contained in several freshwater fish. Nihon Suisan Gakkai-Shi 42: 233–237. (Transl. from Japanese by Fish. Mar. Serv. Transl. Ser. No. 3798, 1976).

Dietzel, K. 1976. Calculation of the prerequisites for the design of a closed sea-water system in stable equilibrium. Mar. Biol. 34: 378–385. (Transl. from German by Fish. Mar. Serv. Transl. Ser. No. 3799, 1976)

Chepurnov, A. V., and N. K. Tkachenko. 1974. Quantitative and qualitative characteristics of lipids in spawning females and progeny during embryonic development of *Gobius melanostomus* from the Sea of Azov. Akad. Nauk Ukr. SSR Ordena Tr. Krasnogo Znameni Inst. Biol. Yuzhn. Morei (Biol. Prod. Yuzhn. Morei). (Transl. from Russian by Fish. Mar. Serv. Transl. Ser. No. 3800, 1976)

Tcheknigovtzeff, C. 1976. Zoology — on the organization of setigerous matrices in two Malacostracan. Crustacea studied under the electron microscope. C. R. Acad. Sci. Ser. D 282: 727–729. (Transl. from French by Fish. Mar. Serv. Transl. Ser. No. 3801, 1976)

Kochi, M. 1976. Distribution of isomeric eicosadienoic acid in lipid classes of a sea urchin. Nihon Suisan Gakkai-Shi 42: 219–225. (Transl. from Japanese by Fish. Mar. Serv. Transl. Ser. No. 3802, 1976)

Yoshimizu, M., T. Kimura, and M. Sakai. 1976. Studies on the intestinal microflora of salmonoids — I. The intestinal microflora of fish reared in fresh water and sea water. Nihon Suisan Gakkai-Shi 42: 91–99. (Transl. from Japanese by Fish. Mar. Serv. Transl. Ser. No. 3803, 1976)

Shimma, Y., H. Ichimura, and N. Shibata. 1976. Effects of starvation on body weight, lipid contents, and plasma constituents of maturing rainbow trout. Nihon Suisan Gakkai-Shi 42: 83–89. (Transl. from Japanese by Fish. Mar. Serv. Transl. Ser. No. 3804, 1976)

Plehn, M. 1906. Peculiar glandular cells in the vascular system and in other organs of fishes. Anat. Anz. 28: 192–203. (Transl. from German by Fish. Mar. Serv. Transl. Ser. No. 3805, 1976)

Popov, A., Ts. Milkova, and N. Marekov. 1975. Free and bound sterols in sunflower, soya and corn oils. Die Nahrung 19: 547–549. (Transl. from German by Fish. Mar. Serv. Tansl. Ser. No. 3806, 1976)

Chugunkov, D. I. 1975. Grey fur seals as an industry. Morsk Mlekopitayushchie. Chast' 2. Mater. IV Vses. Soveshch. p. 167–170. (Transl. from Russian by Fish. Mar. Serv. Transl. Ser. No. 3807, 1976)

Plehn, M. 1906. Glandular cells or parasites? Anat. Anz. 29: 152–156. (Transl. from German by Fish. Mar. Serv. Transl. Ser. No. 3808, 1976)

Seher, A., and H. Vogel. 1976. Study of sterol mixtures IV: the retention behaviour of free sterols in gas chromatography. Fette Seifen Anstrichm. 78: 106–107. (Transl. from German by Fish. Mar. Serv. Transl. Ser. No. 3809, 1976)

Ilsemann, K., I. Reichwald, and K. D. Mukherjee. 1976. Effect of hydrogenation on stability and nutritional properties of lowerucic rapeseed oils. Fette Seifen Anstrichm. 78: 181–187. (Transl. from German by Fish. Mar. Serv. Transl. Ser. No. 3810, 1976)

Anon. 1975. Environmental study on the Romaine–Churchill diversion project. Report and opinion. Dessau Environement Ltee, environment and ecology consultants. 129 p. (Transl. from French by Fish. Mar. Serv. Transl. Ser. No. 3811, 1976)

Beloborodov, A. G., and Yu. K. Timoshenko. 1974. Protection for the Atlantic walrus. Priroda 3: 97–99. (Transl. from Russian by Fish. Mar. Serv. Transl. Ser. No. 3812, 1976)

Stodolnik, L., and Z. Podeszewski. 1975. Lipids in industrial Baltic Sea fish. IV. Lipid fractions in frozen fish under storage. Bromatol. Chem. Toksykol. 8: 9–21. (Transl. from Polish by Fish. Mar. Serv. Transl. Ser. No. 3813, 1976)

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Journal
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Guide for Authors

A more detailed guide on preparation of manuscripts from theses is available from the Office of the Editor.

CONTENT

We invite submission of manuscripts in the broad field of fisheries management, ocean science, and aquatic environments relevant to Canada.

Articles: Reports of research studies of broad scope that are original contributions to science.

Notes: Differ from papers on the basis of scope or completeness, rather than quality of research. They may be on problems with narrow, readily defined limits; on work that is largely confirmatory; on methodology or research techniques; on advances in knowledge arising as by-products of broader studies; on progress in projects that were discontinued; or on preliminary findings that warrant publication before problems are resolved. They may be organized with as much flexibility as articles except that they have no centered headings and should occupy no more than four printed pages.

Reviews: Detailed articles that are critical appraisals of broad areas of investigation, particularly in fields that have shown rapid development in recent years.

Perspectives: Essays of opinion or hypothesis on fisheries, ocean science, aquatic environments, or related topics that are of concern to the professional and layman. References are desirable; informal style is welcomed.

Letters: Opinion on topics recently under discussion in the *Journal*; preferred maximum length, 500 words. The Editor reserves the right to shorten letters and to make other changes to accord with the style of the *Journal*.

EDITORIAL PRACTICES

The *Style Manual for Biological Journals* (3rd ed. American Institute of Biological Sciences, 3900 Wisconsin Ave. NW, Washington, D.C.) is used as the authority in matters of form. The standards for spelling are: *Webster's Third New International Dictionary* and *le Grand Larousse Encyclopédique*. Titles of periodicals are abbreviated as in the *Biological Abstracts* list. References to literature are made by the name-and-year system. The *Journal* follows the names and spelling recommended in *A List of Common and Scientific Names of Fishes from the United States and Canada* (3rd ed. 1970), Special Publication No. 6 of the American Fisheries Society.

Series titles should be avoided. An *abstract* is required for all manuscripts, and should state concisely, in less than 250 words, what was done, the essentials of the new knowledge, and the main conclusions. Abstracts are published in English and French; those submitted in one language only are translated by the *Journal*.

The Editor normally submits each manuscript to at least two *referees*. Contributors are encouraged to suggest at least four names to be added to the list from which referees may be selected.

Illustrations should be submitted as photographic reproductions no larger than a standard page and must accompany the manuscript. Prints should not be mounted, stapled, or clipped. Symbols, letters, and numerals must be neat, and at least 1.5 mm high when the illustration is reduced to the appropriate size. Drawings should, when possible, be grouped to facilitate comparisons.

Proofs are sent to the author, who is responsible for correcting only errors in typesetting. *Alterations to the content must be avoided.*

Fifty *reprints* are supplied free. Additional reprints may be purchased at the time of publication (an order form is sent out with the galley proof). All orders should be sent to the Printing Department, University of Toronto Press, 5201 Dufferin Street, Downsview, Ont. M3H 5T8. Invoices relating to the reprints will be sent out by the Printer. A charge of $40.00 per page is made for articles published. When submitting a manuscript, an author should state that his institution will accept this charge.

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Attention to the following questions will expedite appraisal of manuscripts by referees and editors.

Are the findings, interpretations, and conclusions adequately documented and relevant to the purpose of the study?

Are all the tables and figures needed, and organized to facilitate comparisons? Are there inconsistencies between tables or figures and the text, or within the text? Should some of the data be made available separately, in a manuscript report or at a data repository? If you use statistical analysis are the statistics subordinate to the research?

Would any of the text be clearer if condensed? Are summary statements given at the beginning of sections and paragraphs, and are details in sections and paragraphs relevant to their topics? Does the organization of the manuscript follow logically from the statement of purpose in the introduction?

Does the *title* encompass the content of the report?

Does the *abstract* give the essentials of the new knowledge?

Is the *introduction* largely limited to the scope, purpose, and rationale of the study? Is review of the literature limited to defining the problem?

Are details of *materials and methods* limited to what scientists need in understanding the design of the study and in judging adequacy of the data?

Are generalizations on the *results* adequate? Are findings distinguished from inferences from them?

Is *discussion* limited to interpretation and significance of the findings?

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Guide des Auteurs

On peut se procurer au bureau du Directeur de la publication un guide plus détaillé pour la préparation de manuscrits à partir de thèses.

SUJETS

Nous désirons reçevoir des manuscrits s'insérant dans le vaste domaine de la gestion des pêches, des sciences de la mer et des milieux aquatiques au Canada.

Articles: Comptes rendus d'études d'une portée générale, constituant des contributions inédites à la science.

Notes: Se distinguent des articles par la portée et le degré d'achèvement plutôt que par la qualité des recherches. Elles peuvent traiter de problèmes particuliers et bien définis; de travaux qui sont en grande partie confirmatoires; de méthodologie ou de techniques de recherche; de progrès de la connaissance découlant d'études plus vastes; de développements dans des projets abandonnés; ou de découvertes préliminaires qui méritent d'être publiées avant la solution de problèmes à l'étude. Les notes peuvent être présentées d'une façon aussi souple que les articles, mais elles ne doivent pas porter d'en-têtes centrés ni occuper plus de quatre pages.

Critiques: Articles détaillés constituant une appréciation critique de vastes champs de recherches, particulièrement dans les domaines ayant connu de rapides progrès au cours des dernières années.

Perspectives: Essais d'opinion ou d'hypothèse sur les pêches, les sciences de la mer, les milieux aquatiques, ou sur des sujets connexes qui intéressent professionnels et profanes. Il est préférable de donner des références et d'écrire, si possible, dans un style simple.

Lettres: Opinions sur des sujets exposés dans le *Journal*. Longueur maximale préférable: 500 mots. Le directeur se réserve le droit de raccourcir les lettres et de faire d'autres modifications dans le style du *Journal*.

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Le *Style Manual for Biological Journals* (3e éd., publié par l'American Institute of Biological Sciences, 3900 avenue Wisconsin N.O., Washington, D.C.) sert de guide quant à la forme de présentation. Le *Webster's Third New International Dictionary* et le *Grand Larousse Encyclopédique* font autorité pour l'orthographe des mots. Les titres de périodiques sont abrégés suivant les *Biological Abstracts*. Les références bibliographiques dans le texte doivent suivre le système nom de l'auteur-année. Pour les noms de poissons et leur orthographe, le *Journal* suit les recommandations contenues dans *A List of Common and Scientific Names of Fishes from the United States and Canada* (Troisième édition, 1970), Publication spéciale No. 6 de l'American Fisheries Society.

Il faut éviter les *articles en série*. Un *résumé* doit accompagner chaque texte et doit exposer brièvement (moins de 250 mots) le travail accompli, les points essentiels des connaissances acquises et les principales conclusions. Les résumés paraissent en anglais et en français, le *Journal* veillant à la traduction dans le cas des présentations unilingues.

Le directeur soumet d'ordinaire chaque texte à l'approbation de deux *arbitres* ou plus. Nous encourageons les collaborateurs à suggérer au moins quatre personnes dont les noms pourraient figurer sur la liste des arbitres.

Les *illustrations* doivent être sous forme de reproductions photographiques ne pas dépasser les dimensions d'une page normale et accompagner le texte. Elles ne doivent pas être montées, coupées ni brochées. Les symboles, lettres ou chiffres doivent se détacher avec précision et avoir au moins 1,5 mm de hauteur lorsque l'illustration est réduite à la grandeur voulue. Les dessins doivent, autant que possible, être groupés pour faciliter l'étude comparative.

L'auteur doit revoir les *épreuves* qu'on lui remet et en corriger seulement les erreurs typographiques. *Il doit éviter de changer le contenu du texte.*

Cinquante *tirés à part* sont fournis gratuitement. Les tirés à part peuvent être achetés au moment de la publication. Un bon de commande à cet effet accompagne les épreuves; il doit être retourné en même temps que celles-ci, non pas au bureau de la rédaction, mais au Printing Department, University of Toronto Press, 5201 Dufferin Street, Downsview, Ont. M3H 5T8, qui se chargera de les facturer. Les frais sont de $40.00 par page pour la publication des articles. Quand un auteur soumet un manuscrit, il doit spécifier que son institution accepte les frais.

LISTES DE CONTRÔLES POUR MANUSCRITS

Une attention particulière aux questions suivantes facilitera l'évaluation des manuscrits par les arbitres et les éditeurs.

Les découvertes, interprétations et conclusions sontelles bien documentées et pertinentes au but de l'étude?

Les tableaux et figures sont-ils tous nécessaires et agencés pour faciliter les comparaisons? Y a-t-il contradiction entre les tableaux ou les figures et le texte, ou dans le texte même? Est-ce que certaines données devraient être présentées séparément, dans un rapport manuscrit ou dans un dépôt de données? Si vous faites une analyse statistique, la statistique cède-t-elle le pas à la biologie?

Le texte y gagnerait-il en clarté s'il était plus condensé, en tout ou en partie? Contient-il des exposés sommaires au début de chaque section ou paragraphe, et les détails de ces sections et paragraphs son-ils pertinents au sujet traité? La marche du manuscrit est-elle conforme à la logique énoncée dans l'introduction?

Le *titre* est-il concis, tout en étant représentatif du contenu du rapport?

Le *résumé* donne-t-il l'essentiel des connaissances nouvelles?

L'*introduction* se limite-t-elle à une esquisse de la portée, du but et des raisons de l'étude? La revue de la littérature se limite-t-elle à la définition du problème?

La section *matériel et méthode* contient-elle le minimum de détails qui permettront aux autres scientifiques de saisir les grandes lignes de l'étude et de juger de la valeur des données?

Les généralisations sur les *résultats* sont-elles adéquates? Permettent-elles de distinguer entre constatations et déductions?

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Guide for Typists Preparing Copy

Read "Guide for Authors" in the current volume of the *Journal*. When in doubt about how to type or arrange details, follow *Journal* practice.

Type the manuscript on white bond paper, not flimsy, preferably size 21.5 × 28 cm (8.5 × 11 inches). If possible, use paper with the lines numbered in the margin. Leave all margins at least 2.5 cm wide.

Double-space all parts of the manuscript, including tables, captions for illustrations, footnotes, and the reference list. Underline only material that is to be printed in italics. Indent the first line of each paragraph in the text and of each footnote.

In titles and headings, capitalize the first letter of each word. For small capitals, use small letters.

Indicate all text footnotes by superscript arabic numerals. Number them consecutively throughout the manuscript, starting with those on the title page. Place each footnote immediately below the reference to it, separating it from the text by horizontal lines.

On the first page type only the title, the authors' names and affiliations, related footnotes, and the running head (see Appendix A). Type the abstract on a separate page (see Appendix B). Number all pages, beginning with the title page and including those for tables and captions for illustrations.

Have the author check the finished manuscript, and send the ribbon copy and two other clear copies to the Office of the Editor.

Tables: Examine the current volume of the *Journal* to see how tables are set up (see Appendix C).

Note especially the following concerning *numbered tables:*

Type each on a separate page, using paper without the lines numbered.

Number them with arabic numerals.

Use horizontal lines above and below the headings and below the columns, and seldom elsewhere. Never use vertical lines; leave extra spaces instead.

Indicate table footnotes by superscript small letters and type them below the table.

Place the tables after the list of references.

Where each table is first referred to, type in the margin "Table — near here."

Note that *text tables* are not numbered, are typed with the text, and seldom need horizontal lines (see Appendix C).

Illustrations: Send a photographic reproduction (no larger than 21.5 × 28 cm) of each illustration with the manuscript. Type the author's name and figure number on a strip of paper and fasten it to the back of the print. Do not write or type on the print itself. Also, type the captions in succession on a separate page, headed "Captions for illustrations." Put this page at the end of the manuscript.

Where each figure is first referred to, type in the margin "Fig. — near here."

Mathematical Expressions, Equations, and Formulas: Leave three spaces above and below lines that have much material in superscripts, and above and below equations and formulas. In the various expressions, place each component in its correct position (see Appendix D).

Show all symbols clearly. In the margin, spell out Greek letters the first time they occur, and distinguish between the small letter *l* and the numeral *one*, and between the capital letter *O* and the numeral *zero*. Make other explanatory notes similarly.

References: Check the text to see that all references in the list are referred to. Type the reference list, double-spaced, in the style used in the *Journal* (see Appendix E). Names of journals are abbreviated as in the *Biological Abstracts* list; if an abbreviation is in doubt, type the name in full.

Spellings: About 100 words common in fisheries journals are listed in Appendix F.

Appendices: Appendices A to F are available on request from the Office of the Editor.

Journal
de l'office
des recherches
sur les pêcheries
du Canada

Guide pour les secrétaires d'auteurs

Lisez le "Guide des Auteurs" reproduit dans l'édition courante du *Journal*. Si vous avez des doutes sur la façon de dactylographier le texte ou d'en agencer les détails, suivez la pratique du *Journal*.

Dactylographiez le manuscrit sur papier blanc de bonne qualité, et non sur papier pelure, mesurant de préférence 21,5 × 28 cm (8,5 × 11 pouces), laissant toujours une marge d'au moins 2,5 cm. Si possible, employez un papier avec lignes numérotées en marge.

Tout le manuscrit, y compris tableaux, légendes de figures, renvois et références bibliographiques, devra être dactylographié à double interligne. Ne soulignez que les mots qui devront être imprimés en italique. Mettez en retrait la première ligne de chaque paragraphe du texte et de chaque renvoi.

Dans les titres et les en-têtes, n'employez la majuscule que pour le premier mot et les noms propres. Pour les petites majuscules, employez les miniscules du dactylographe.

Indiquez tous les renvois du texte par des chiffres arabes suscrits. Numérotez les renvois consécutivement par tout le texte, à commencer par ceux de la page titre. Placez chaque renvoi directement sous sa référence, le séparant du texte par des lignes horizontales.

La première page ne doit contenir que le titre de la publication, le nom et l'affiliation des auteurs, les renvois appropriés et le titre courant (voir Appendice A). Dactylographiez le résumé sur une page séparée (voir Appendice B). Numérotez chaque page, à commencer par la page titre, y compris celles qui contiennent les tableaux et la légende des figures.

Voyez à ce que l'auteur vérifie le manuscrit final, et soumettez la première copie ainsi que deux copies lisibles au bureau du Directeur de la publication.

Tableaux. Consultez une copie du *Journal* pour la composition des tableaux (voir Appendice C).

Portez une attention particulière à ce qui suit au sujet des *tableaux numérotés.*

Dactylographiez chaque tableau sur feuille séparée, vous servant de papier sans lignes numérotées.
Numérotez les tableaux en chiffres arabes.

Tracez des lignes horizontales au-dessus et au-dessous des en-têtes et au-dessous des colonnes, rarement ailleurs; ne tracez jamais de lignes verticales; laissez plutôt des espaces supplémentaires.

Indiquez les renvois dans les tableaux par les minuscules suscrites et placez les renvois au bas du tableau.
Placez les tableaux à la suite des références bibliographiques.
A l'endroit où le tableau est mentionné pour la première fois, indiquez dans la marge "Tableau — près d'ici."

Remarquez que les *tableaux dans le texte* ne sont pas numérotés, sont dactylographiés avec le texte et ne requièrent que rarement des lignes horizontales (voir Appendice C).

Figures: Envoyez avec le manuscrit un reproduction photographique (21,5 × 28 cm ou moins) pour chaque illustration. Dactylographiez le nom de l'auteur et le numéro de la figure sur une lisière de papier que vous collerez au dos. N'écrivez pas à la main ou à la machine sur la photographie même. En plus, dactylographiez les légendes, dans l'ordre, sur feuille séparée, intitulée "Légendes des figures." Intercalez cette feuille à la suite du texte.

A l'endroit où chaque figure est mentionnée pour la première fois, inscrivez en marge "Fig. — près d'ici."

Equations, formules et expressions mathématiques: Laissez trois espaces au-dessus et au-dessous des lignes renfermant des chiffres ou des lettres suscrits et souscrits, ou encore au-dessus et au-dessous d'équations et de formules. Placez chaque composante des différentes expressions dans la position qui lui est propre (voir Appendice D).

Indiquez clairement tous les symboles. Epelez en marge les lettres grecques, la première fois qu'elles apparaissent. Faites une distinction entre la lettre *l* et le chiffre *un*, et entre la majuscule *O* et le chiffre *zéro*. Insérez de la même façon toute autre note explicative.

Références: Vérifiez le texte afin de vous assurer que toutes les références apparaissant dans la liste bibliographique sont bien mentionnées dans le texte. Dactylographiez à double interligne dans le style en usage dans le *Journal* (voir Appendice E). Les noms de revues sont abrégés suivants les *Biological Abstracts*; en cas de doute, écrivez le nom au long.

Orthographe: Une liste d'environ 100 mots communément employés dans les périodiques sur les pêches est donnée en Appendice F.

Appendices: On peut se procurer une copie des Appendices A à F sur demande au bureau du Directeur de la publication.

Canadian Conference For Fisheries Research

The thirtieth Annual Meeting of the CCFFR will be held at the Lord Beaverbrook Hotel, Fredericton, New Brunswick, January 5 and 6, 1977. The Fisheries Research Board of Canada and the Fisheries and Marine Service, Department of the Environment, will again sponsor this meeting. The President is J. M. Anderson, University of New Brunswick, Federicton, the Secretary is J. C. Stevenson, and the Treasurer is J. Camp, both of the Fisheries and Marine Service, Department of the Environment, Ottawa. Program Chairman is F. W. H. Beamish, University of Guelph, Guelph.

The Canadian Society of Environmental Biologists will also meet at Fredericton, January 4, 1976.

CCFFR Program

Wednesday January 5 0900–1200 h

Contributed Papers Session

Convenor:
P. M. Powles
Department of Biology
Trent University
Peterboro, Ont.

Persons intending to present papers should contact the Convenor at the earliest possible date providing the proposed title and, if possible, an abstract of the paper.

Wednesday January 5 1400–1700 h

Implications of the 200-Mile Zone on Fisheries Research and Management

Convenor:
A. W. May,
A/Director-General
Resource Services Directorate
Fisheries Management
Ottawa, Ont.

1. Development of a 200-Mile Zone in Law of the Sea
   — Speaker to be confirmed.
2. Management of Pacific Salmon Relative to the 200-Mile zone
   J. MacDonald, Fisheries and Marine Service, Nanaimo, B.C.
3. Atlantic Groundfish Management Relative to the 200-Mile Zone
   L. S. Parsons, Fisheries and Marine Service, Ottawa, Ont.
4. Fisheries Management Concepts and Demands on the Knowledge Base
   B. S. Muir and A. W. May, Fisheries and Marine Service, Ottawa, Ont.

Thursday January 6 0900–1200 h

Changing Patterns of Utilization of Sea Mammals

Convenor:
D. E. Sergeant
Environment Canada
Arctic Biological Station
P.O. Box 400
Ste. Anne de Bellevue, Que.

1. Population Changes in Harp Seals under Exploitation
   D. M. Lavigne, Department of Zoology, University of Guelph, Guelph, Ont.
2. Atlantic Coast Seal Populations and Their Management
   J. Boulva, Laval University, Quebec, Que.
3. West Coast Killer Whales
   M. A. Bigg, Department of the Environment, Pacific Biological Station, Nanaimo, B.C.
4. White Whales in the St. Lawrence; Indirect Effects of Man on Populations
   J. Laurin, Department of Zoology, University of Guelph, Guelph, Ont.

Thursday January 6 1400–1700 h

Embryonic and Larval Periods of Fishes: Present Status and Significance

Convenor:
E. K. Balon
Department of Zoology
University of Guelph
Guelph, Ont.

A panel discussion with the following members:

D. F. Alderdice*, Department of the Environment, Pacific Biological Station, Nanaimo, B.C.
H. Battle*, Department of Zoology, University of Western Ontario, London, Ont.
D. Faber, National Museums of Canada, Ottawa, Ont.
P. Ihssen, Ontario Ministry of Natural Resources, Maple, Ont.
A. E. Peden*, B.C. Provincial Museum, Victoria, B.C.
C. C. Lindsey, Department of Zoology, University of Manitoba, Winnipeg, Man.

*Not yet confirmed.

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